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PART 1

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Indiana Junior Academy of Science Council: DONALD R. WINSLOW, 1975, Chairman; SR. MARY ALEXANDRIA, 1972; MARY J. PETTERSEN, 1973; LAWRENCE POORMAN, 1974; HELEN REED, 1971.

Library Committee: LOIS BURTON, Chairman; NELLIE COATS; WILLIAM R. EBERLY; JOHN W. KLOTZ; Eli Lilly.

Program Committee: CARROLLE MARKLE and WILLIAM STEPHENSON, Co-Chairmen.

Publications Committee: MARION T. JACKSON, Chairman; MARION F. BAUMGARDNER; HARRY G. DAY; WILLIAM R. EBERLY; WILTON N. MELHORN; JOHN F. PELTON; B. K. SWARTZ, JR.

Newsletter: FRANK A. GUTHRIE.

Relation of the Academy to the State of Indiana: WILLIAM A. DAILY, Chairman; JAMES A. CLARK; WILLIAM R. EBERLY; HELMUT KOHNKE.

Membership Committee: MARVIN SCHREIBER, Chairman; ELIOT C. WILLIAMS.

Fellows Committee: BEN MOULTON, 1973, Chairman; JAMES A. AHLRICHS, 1973; CHARLES E. BRAMBEL, 1973; RICHARD L. CONKLIN, 1971; SEARS CROWELL, 1972; FAY K. DAILY, 1971; WILLIAM W. DAVIS, 1973; ROBERT E. GORDON, 1972; CHARLES B. HEISER, 1971; DONALD E. MILLER, 1971; B. E. MONTGOMERY, 1972; GEORG K. NEUMANN, 1972; JOHN PELTON, 1972.

Resolutions Committee: ARTHUR T. GUARD, Chairman; HOWARD R. YOUSE, ROBERT O. PETTY.

Invitations Committee: JAMES R. GAMMON, Chairman; ALTON A. LINDSEY; JAMES C. LIST; PAUL R. QUINNEY; WILLIAM K. STEPHENSON.

Parliamentarian: PAUL WEATHERWAX.

SPECIAL COMMITTEES APPOINTED BY THE PRESIDENT

Biological Survey Committee: LELAND CHANDLER, Chairman; JACK E. HUMBLE; GAYTON C. MARKS; RUSSELL E. MUMFORD; JACK R. MUNSEE; WINONA H. WELCH; FRANK N. YOUNG.

Emeritus Member Selection Committee: RALPH E. CLELAND, Chairman; ROBERT H. COOPER, EDWARD L. HAENISCH; HOWARD MICHAUD; WINONA H. WELCH; J. DAN WEBSTER.

Preservation of Scientific Areas Committee: ROBERT O. PETTY, Chairman; RAY GUTSCHICK; CARL H. KREKELER; CARROLLE MARKLE; BEN MOULTON; DAMIAN V. SCHMELZ; ROBERT WEBER; WINONA H. WELCH.

"Speaker of the Year" Selection Committee: RALPH E. CLELAND, Chairman; WILLIS H. JOHNSON; A. J. ULLSTRUP.

Science and Society Committee: WILLIS H. JOHNSON, Chairman; RALPH E. CLELAND; HARRY G. DAY; WILLIAM R. EBERLY; ALTON A. LINDSEY; PAUL E. KLINGE; ROBERT D. MILES; ROBERT MENKE; ROBERT E. GORDON; MILTON BURTON; JOHN B. PATTON; D. JAMES MORRE; HERMAN B. WELLS, Honorary; HELMUT KOHNKE, Executive Secretary.

Member, Indiana Natural Resources Commission: SAMUEL N. POSTLETHWAIT.

SPRING MEETING

MARY GRAY BIRD SANCTUARY, Fayette County, Indiana

MINUTES OF THE EXECUTIVE COMMITTEE MEETING

April 23, 1971

The meeting was called to order at 4:39 PM by President Samuel Posthlethwait in Earl Brooks Hall, Mary Gray Bird Sanctuary, near Connersville. Nineteen members were present. The president reported briefly, chiefly noting points of the agenda which were considered later in the meeting. The secretary moved the approval of the minutes of the November 5, 1970, meeting, which had been duplicated and distributed. Seconded and carried.

Treasurer: Rev. Damian V. Schmelz distributed duplicated copies of his report for the period January 1 to April 23, 1971. It was moved, seconded, and carried that it be accepted.

Academy Foundation Committee: William A. Daily, Chairman, reported that legal counsel had been retained to clarify the tax status of the academy with the United States Internal Revenue Service. He moved that attorney Donald G. Sutherland be granted Power of Attorney to act for the academy in tax matters. Seconded and carried. (This action had previously been taken by the Council.)

Bonding Committee: Robert M. Brooker, Chairman, recommended no change in our program.

Academy Representative on the A.A.A.S. Council: Willis H. Johnson, Representative, reported the following interesting actions at the December, 1970, meeting of the council: 1) Passed resolution recommending phaseout of the use of herbicides in Southeast Asia; 2) Passed resolution recommending that all scientists start using the metric system in their publications and also recommending that the government establish a national policy on its use; 3) Passed resolution reaffirming its commitment to equal opportunity for all scientists regardless of sex, race, creed or national origin.

A new rule of the A.A.A.S. requires representatives from affiliated organizations (such as ours) to serve 2-or-more-year terms. It was moved that the constitution of the academy be amended as follows: (italicized words added)

"Article V, Section 1. The following named committees shall be appointed by the President, to serve for one year, *except as noted otherwise below and* except as rotation is provided, and except as otherwise provided *they* shall be announced . . .

"(1) The Academy Representative on the Council of the American Association for the Advancement of Science. *This person shall serve for a three-year term beginning January 1, 1972, and every 3 years thereafter.*

"(2) Auditing. . ." Seconded and carried.

Auditing Committee: Robert Dolphin, Chairman, stated that no report was necessary at this time of year.

Library Committee: Mrs. Lois Burton, Chairwoman, reported that in December the Academy received a grant of \$15,000 from Lilly Endowment, Inc., to be expended in general support of the John Shepard Wright Memorial Library. The amount is on the Treasurer's books as Lilly Fund IV. We plan to begin carrying out provisions under this grant during the coming summer.

A second mailing of Volume 79 of the *Proceedings* has been completed, courtesy of the Indiana State Library. The total number mailed to members to date is 1,127.

Monograph Number 2 has been received and is ready for mailing under our Exchange program to libraries, universities, and scientific societies.

Some sales of *Natural Features of Indiana* were made. Sixty-five copies remain in stock.

Program Committee: Mrs. Carroll A. Markle reported no problems.

Publications Committee: Marion T. Jackson, Editor, reported that Monograph 2 has been published and will be distributed free to members who request it, pending investigation of costs and past actions of this committee. Volume 80 of the *Proceedings* will be published about September and will be larger than the last volume. The possibility of page charges or of a limitation of allowable published papers per year per member should be considered for future volumes.

Relation of Academy to State of Indiana: William A. Daily, Chairman, reported that no change had been made in the State's budget allowance for publication of the *Proceedings*.

Membership Committee: Eliot C. Williams, Chairman, reported no radical changes.

Invitations Committee: The secretary reported official approval from Indiana Central College of that school as our meeting site for 1973.

Emeritus Members Committee: The secretary read a report from Chairman Ralph Cleland which recommended that Ralph W. Lefler be elected to emeritus status. It was moved, seconded, and carried that Dr. Lefler be so elected.

Natural Areas Committee: Rev. Damian V. Schmelz reported that the registry of natural areas in the state has been extended, intensified, and more effectively computerized.

Speaker of the Year Committee: The committee consisted of Ralph F. Cleland (chairman), Willis H. Johnson (reporting), and Arnold Ullstrup. Willis Johnson moved that,

The Special Lecturer should be paid an honorarium of \$500.00 by the Academy.

A committee should select the Special Lecturer each year. While the Executive Committee might do this and the contacting of the colleges, you may prefer to appoint a special committee for this purpose. If you decide on a special committee of three, each should serve three years except in the beginning when one should serve for three years, one for two, and one for one. Thus, after the first year one new member would be appointed by the President each year.

The job of the committee would be two-fold: 1) to select the speaker, and 2) to contact the colleges.

The speaker should be a member of the Academy (preferably). He should be a forceful speaker, able to communicate with young people. His subject should be relevant. It might be on his research, but it should be related to social problems. On the other hand, it might be on a social problem or problems not directly related to his research. The committee should know the subject of a prospective speaker before he is selected. It is understood that the speaker will give his lecture before the fall meeting of the Academy, preferably in a session immediately following the lunch period. This lecture will be printed in the *Proceedings of the Indiana Academy*. In addition to the lecture before the Academy, the speaker will give his lecture at four or five colleges in the state. It is understood that this is a program for the colleges of the state. The second job of the committee each year will be to contact the colleges where the speaker will give his lecture. Once a college has been served it should not be contacted again for a special lecturer until all of the colleges of the state have had an opportunity to have a special lecturer. It may be that the committee will want to select colleges in a particular region of the state for a given year. It is understood that the colleges will pay for any meals and lodging involved in the visit; the colleges will not pay an honorarium.

Seconded and carried.

Willis H. Johnson moved that the speaker of the year for 1971 be Alton A. Lindsey, with first alternate William R. Breneman and the second alternate Charles B. Heiser, Jr. Seconded and carried.

Science and Society Committee: Willis H. Johnson, Chairman, reported that the committee and its Executive Director, Helmut Kohnke, had been able to advise several branches of the state and city governments concerning environmental and other scientific problems.

The President reported that a group of engineers at Purdue University had requested the formation of an Engineering Section of the Academy. It was moved by Rev. Damian V. Schmelz, seconded, and carried, that an Engineering Section be organized at the 1971 fall meeting; that the section follow all appropriate procedures and restrictions consistent with the constitution and by-laws of the Academy. The President appointed Robert L. Swaim Chairman for 1971.

Marion T. Jackson reported the recent death of Georg K. Neumann, who was the elected 1971 chairman of the Anthropology Section. The President appointed William R. Adams Chairman for the remainder of the year.

There was an extended discussion of some recent attributions by news media of "official Indiana Academy of Science" positions to public statements by certain members. Mrs. Fay K. Daily moved that the Parliamentarian draft a disavowal statement for publication by the Academy, disassociating the academy officially from all public statements by its members. The motion was seconded; a vote by show of hands was tied. It was moved by Willis H. Johnson, seconded, and carried that the motion be tabled until next meeting.

The President requested an investigation of the possibility of hiring an Executive Secretary. No objection being raised, consent was declared.

The President stated:

"The President of the Academy by law is the official appointee to the Natural Resources Commission. It is believed that it would serve the best interests of the Academy and the Natural Resources Commission if the law were changed to permit the appointee to be the president or his representative."

It was moved by Willis H. Johnson, seconded, and carried that the Academy petition the Indiana State Natural Resources Commission and the legislature of the State of Indiana to change the law so that the member of the Natural Resources Commission not be "The President of the Indiana Academy of Science", but, rather, "The President of the Indiana Academy of Science *or his designated representative.*"

On motion the meeting adjourned at 6:32 PM.

Approved October 28, 1971.

Respectfully submitted,
J. DAN WEBSTER, Secretary

MINUTES OF THE GENERAL SESSION

April 23, 1971

The meeting was called to order by President Samuel N. Postlethwait at 7:49 PM in Earl Brooks Hall, Mary Gray Bird Sanctuary, near Connersville. Thirty members were present.

The secretary moved the following proposed amendment to the by-laws of the Academy—

Article III, Section 1. Delete the last clause, reading, "provided that any bill for fifty dollars or more shall be approved by the president." As a result, Article III, Section 1, would read: "The treasurer is authorized to issue checks in payment of bills submitted by officers or committees for which provision has been made in the budget for that year."

Seconded and carried. (This was the second passage; the amendment is now effective.)

A. T. Guard moved the following resolution—

The Indiana Academy of Science members and friends here assembled express their most sincere appreciation to Dr. Carrolle A. Markle and Dr. W. K. Stephenson for the fine program which they have arranged. Our sincere thanks are extended to the officials of the Indiana Audubon Society and especially to Mr. and Mrs. Denzil Barricklow, Resident Naturalists of the Mary Gray Bird Sanctuary, for making their facilities available to us.

Our thanks also go to Dr. Gary Barrett of Miami University for his timely, instructive and interesting address on "The New Ecology".

We also wish to express our appreciation to those ladies who served us such excellent meals.

Our appreciation is extended to the leaders of the various field trips which are always such an integral part of the spring meeting of the Academy.

Seconded and carried.

Dr. Carrolle A. Markle announced details of field trips and meals. The evening feature was an illustrated lecture by Gary Barrett entitled "The New Ecology".

The meeting adjourned at 9:15 PM.

Approved October 28, 1971.

Respectfully submitted,
J. DAN WEBSTER, Secretary

FALL MEETING

EARLHAM COLLEGE, Richmond, Indiana

MINUTES OF THE EXECUTIVE COMMITTEE MEETING

October 28, 1971

The meeting was called to order at 7:47 PM by President Samuel N. Postlethwait in the Orchard Room of Runyan Center, Earlham College. Twenty-two members were present at the start; ten more came in later. The secretary moved the approval of the minutes of the executive committee and general session meetings of April 23, 1971, as duplicated and distributed. Seconded and carried.

Treasurer: Rev. Damian V. Schmelz presented his report for the period January 1 through October 28, 1971, as follows:

Academy accounts

Income	\$ 5,759.03
Expenditure	5,865.49
Net loss	106.46
Balance December 31, 1970	10,296.09
Balance October 28, 1971	10,189.63

Administered accounts

Income	39,586.79
Expenditure	47,005.93
Net loss	7,419.14
Balance December 31, 1970	43,920.55
Balance October 28, 1970	36,501.41

It was moved by the Treasurer, seconded, and carried that the report be accepted. (On all following committee reports, action, unless noted otherwise, was accepted by general consent.)

Bonding Committee: Robert M. Brooker, Chairman, wrote in, recommending no change in our program.

Director of Public Relations: Paul Klinge wrote in, reporting excellent press coverage during the past year.

Research Committee: James E. Newman, Chairman, reported the following grants in 1971:

J. Dan Webster (Hanover College). Breeding bird censuses in Indiana old growth forests.\$ 378.

Robert E. Pace (Indiana State University). Aerial survey of archeological sites in the Lower Wabash Valley.\$ 505.

Paul T. McKelvey (Tippewa Tech. Institute). Study of water quality of the Tippecanoe River.\$ 530.

D. James Morré (Purdue University). Chlorinated hydrocarbons and egg shell changes.\$ 500.

Winona H. Welch (DePauw University). Hookeriaceae in the West Indies	\$ 200.
Damian V. Schmelz (St. Meinrad College). A comparative study of the physical environment and biotic elements of three strip-mine lakes	\$ 299.
Charles E. Mays (DePauw University). To continue a study of salamander and turtle populations on the North Fork River in Ozark County, Missouri.	\$ 450.
Total grants approved	\$2,862.

Also, one more proposal is under consideration and another from last year has been extended. A manuscript by Sherman Minton, "*Reptiles and Amphibians of Indiana*," has been approved by the Publications Committee as *Monograph No. 3*; the research committee will consider underwriting the publication cost.

Academy Representative to the A.A.A.S. Council: Willis H. Johnson reported no actions by the council relevant to our Academy.

Youth Activities Committee: A written report by Paul E. Klinge, Chairman, was presented by Donald R. Winslow. The anti-animal-experimentation rule set up by the National Science Talent Search still hampers Indiana student biologists. Reorganization of the entire youth program is under discussion, but no proposals are ready.

Science Talent Search Committee: A report was written in by Wendell F. McBurney, Director. The report was accepted, but no action was taken on the request to support the position of the Indiana Science Talent Search Committee regarding experimentation on vertebrate animals.

Science Fairs: A report was written in by Karl Kaufman, director.

Indiana Junior Academy of Science: Donald R. Winslow presented his report and distributed copies of the program for the forthcoming Saturday meeting. It appears that a valuable and interesting meeting for high school students is scheduled. It will include a report on a successful high school applied ecology program, six invited research reports by high school students, an address by Lawrence E. Poorman, and a tour of the Earlham College research facilities.

Library Committee: Mrs. Lois Burton, Chairwoman, reported that 600 square feet of additional floor space have been obtained, but without the needed shelving. Four hundred volumes have been bound. Sixty-six additional journals have been received as exchange; more are being sought.

Trustees of the Academy Foundation: William A. Daily gave this annual report, as of September 30, 1971—

I. Foundation fund	
Last year's balance	\$ 979.96
Receipts	897.05

Disbursements	300.00
Balance	536.57
Market value of securities held	\$ 19,472.00
II. John S. Wright fund	
Last year's balance	\$ 16,627.99
Receipts	16,917.19
Disbursements	14,590.11
Balance	18,955.07
Market value of securities held	\$596,440.00

Program Committee: Mrs. Carrolle A. Markle, Co-chairman, reported that no serious problems were handicapping her committee.

Publications Committee: Marion T. Jackson, Editor and Chairman, reported. The cost of the current Volume 80 of the *Proceedings* now being distributed, has risen sharply over preceding years, but no final figure is yet available. The size increase was 9% in the divisional reports section; had there not been a more careful review system initiated, this increase would doubtless have been much greater. The 10-year index is due in Volume 81.

Frank A. Guthrie moved that *Monograph No. 2* and all subsequent monographs be sold to members at one-half price; it was seconded and carried. (The vague action on the same subject taken April 23, 1971—see minutes—is herewith rescinded.)

Newsletter: Frank Guthrie, Editor, reported that the last issue came out in December, and that the next will be published in November 1971.

Relation of Academy to the State of Indiana: As it has for many years, the legislature in 1971 appropriated \$4,000 per year for the next two fiscal years to aid printing of the *Proceedings*.

Fellows Committee: Benjamin Moulton, Chairman, moved the election of these 7 members to the rank of Fellow of the Academy—

Robert E. Dolphin	Holm W. Neumann
John D. Favinger	B. K. Swartz, Jr.
John W. Hart	Gertrude L. Ward
Edward V. McMichael	

The motion was seconded and carried.

Invitations Committee: A report was written in by James R. Gammon, Chairman. We will meet at St. Mary's College in 1972, at Indiana Central College in 1973, and at DePauw University in 1974.

Preservation of Scientific and Natural Areas Committee: Robert O. Petty, Chairman, reported that a current listing of 429 areas and their outstanding features is on magnetic computer tape and available on request.

Speaker of the Year Committee: Willis H. Johnson, Chairman *pro tem*, reported that the list of colleges where speaker of the year Alton A. Lindsey would appear was under consideration.

Science and Society Committee: The death of Ralph F. Cleland has been acutely felt by the committee.

A booth which was operated with much effort at the recent state fair resulted in some publicity.

Special Committee on Executive Secretary: Jerry Nisbet, Chairman, reported that his committee had decided that the idea of having an Executive Secretary was valid, but expensive. At present, it is beyond our means unless we can find a considerable new source of funds.

When the President called for old business, Mrs. Fay K. Daily called for her tabled motion from the April 23, 1971 meeting. (See page 9.) Mrs. Daily stated that her motion should read: "The Indiana Academy of Science shall not be responsible for public statements of opinion of its members concerning governmental or political matters." After further discussion, the motion was put to a vote by show of hands; it was defeated. In discussion, all were agreed that the Academy must be chary of expressing opinions on political or governmental matters officially. However, public expressions by members, or even officers, not based on official actions may be reported in the public news media as the news media prefer. Alton A. Lindsey moved that any statements made by him, as Speaker of the Year, in 1971-72 be considered individual opinions and not official positions of the Academy. Seconded and carried.

New business was called for. William J. Brett suggested the establishment of an animal behavior section, or at least the investigation of such a possibility for next year. Rev. Damian V. Schmelz moved that the annual dues schedule be increased to: Regular \$6, Family \$8, Student \$3, with no change in the initiation fee; the motion was seconded. Frank A. Guthrie moved to amend so that the action was merely a recommendation to the budget committee and council at their forthcoming December 4, 1971 meeting; the amendment was seconded and carried. Helmut Kohnke moved to amend to increase the recommended dues schedule to \$8, \$10, and \$4 but abolishing the initiation fee; this amendment was seconded, but defeated. The original motion was carried as amended.

A showing of his pictures from the Galapagos Islands by Robert Cooper after the close of the meeting was announced.

The meeting adjourned on motion at 10:39 PM.

Approved April 28, 1972.

Respectfully submitted,
J. DAN WEBSTER, Secretary

GENERAL SESSION—OCTOBER 29, 1971

RICHMOND, INDIANA

President Postlethwait called the meeting to order at 2:07 PM in Wilkinson Theater of Runyan Center, Earlham College. Provost Helen Hole welcomed us to Earlham College in a few gracious words. The secretary read a brief summary of the actions of the executive committee at its meeting last night, and moved its acceptance. Seconded and carried. The secretary moved approval of the following amendment to the constitution; which had been passed by the executive committee on April 23, 1971:

Article V, Section 1. The following named committees shall be appointed by the President, to serve for one year, *except as noted otherwise below and except as rotation is provided, and except as otherwise provided they shall be announced . . .*

(1) The academy representative on the council of the American Association for the Advancement of Science. *This person shall serve for a three-year term beginning 1 January 1972 and every 3 years thereafter.*

(2) Auditing . . .

(added words italicized)

Seconded and carried.

Mrs. Fay K. Daily named the members of the Academy who had died during the past year. (See Necrology Report, page 27.) A moment of silence was observed in their honor.

As speaker of the year, Dr. Alton A. Lindsey gave an outstanding lecture, "The Role of Scientists in the Preparation and Evaluation of the New Environmental Impact Statement Requirement." Recent history of the federal government shows encouraging new laws for protecting the environment, much lip service to conservation by many politicians, but little real protection of the environment.

The meeting was temporarily adjourned at 3:15 PM.

The meeting was recalled to order at 8:08 PM, after dinner in the Friend's Room of Earlham Hall by President-elect Behrens. The secretary read these results of the elections of chairman in the various sections:

Section	1972 Chairman	1973 Chairman-elect
Anthropology	Edward Dolan	
Botany	Willard F. Yates	C. L. Gehring
Cell Biology	Charles W. Goff	
Chemistry	Richard Pilger	William Nevill
Ecology	Alton A. Lindsey	
Engineering	Robert Swaim	
Entomology	Claude F. Wade	Walter J. Weber

Section	1972 Chairman	1973 Chairman-elect
Geography-Geology	Richard L. Powell	Arther Mirsky
Micro-and Molecular Biology	Morris Pollard	
Physics	Torsten Alväger	Peter Skadron
Plant Taxonomy	Gerald Gastony	Ted Crovello
Science Education	Frederick K. Ault	Jack Brewer
Soil Science	Christian Johannsen	William R. Gommel
Zoology	Dorothy Adalis	

Dr. Arthur T. Guard moved the following resolution—

Members of the Indiana Academy of Science, here assembled, wish to express our sincere thanks to Earlham College for opening their facilities to us for our meetings. We are also grateful to Provost Helen Hole for her warm words of welcome. Our thanks also go to Dr. A. A. Lindsey for his timely, instructive and interesting address. We extend our appreciation to Carrolle A. Markle and William K. Stephenson, co-chairmen of the program committee, for their careful planning of the many details associated with our meeting and also to the chairmen of the various divisions! meetings that make the fall meeting both stimulating and instructive.

It was seconded and carried. The resolutions committee consisted of Robert O. Petty, Howard R. Youse, and A. T. Guard, Chairman.

The nominating committee (Harry G. Day, Chairman, A. T. Guard, and Frank A. Guthrie) presented the following slate of candidates for office—For President-elect, William B. Hopp. For Treasurer (1972-75), Clyde R. Metz. For Trustee of the Academy Foundation, Damian Schmelz. For Bonding Committee, Robert Brooker and John A. Ricketts. For Research Grants Committee (1972-76), Kenneth E. Nichols. A. T. Guard moved that these men be nominated and that we cast a unanimous ballot for their election. Seconded and carried.

Samuel Postlethwait presented a study of his methods of teaching botany, entitled, "Individualized Study for Today's Student." It was thought-provoking for all teachers, and well illustrated by movies and still photographs.

The meeting adjourned, on motion, at 9:15 PM.

Approved April 28, 1972.

Respectfully submitted,
J. DAN WEBSTER, Secretary

FINANCIAL REPORT OF THE INDIANA ACADEMY OF SCIENCE

JANUARY 1—DECEMBER 31, 1971

I. ACADEMY ACCOUNTS

	Income	Expenditure	Budgeted
Dues	\$ 3,866.00		
Interest	2,227.39		
Secretary		\$ 421.30	\$ 500.00
Clerical			
Postage, etc.	\$ 421.30		
Treasurer		233.15	225.00
Clerical	29.00		
Postage, etc.	204.15		
Office		143.45	175.00
Travel, AAAS Dues		171.30	180.00
Transfer to Adm. Accts.		2,775.00	
Proceedings: Pub.	500.00		500.00
Proceedings: Mailing	100.00		100.00
Library Binding	1,000.00		1,000.00
Science & Society	600.00		600.00
Natural Areas	575.00		575.00
President's Fund			100.00
Membership Comm.			75.00
Junior Academy		104.37	200.00
Program Committee		707.18	600.00
1970 Meetings			
Chairman	20.00		
Printing	214.00		
1971 Meetings			
Chairman	174.29		
Printing	239.79		
Mailing	59.10		
Publications: Editor		400.00	400.00
Speaker of the Year			
Honorarium		500.00	500.00
Administrative			50.00
Newsletter		105.68	100.00
	\$ 6,093.39	\$ 5,561.43	\$ 5,880.00
Reprints: Vol. 78	270.00		
Vol. 79	706.55		
Vol. 80	1,730.70	2,790.70	
Miscellaneous		3,538.49	
Attorney Fees	3,460.49		
Bonding Fee	63.00		
Public Relations	15.00		
	\$ 8,800.64	\$11,890.62	

II. ADMINISTERED ACCOUNTS

	January 1 Balance	1971 Income	1971 Expenditure	December Balance
Science Talent	\$ 1,834.37	\$ 1,600.00	\$ 1,587.73	\$ 1,846.64
Science Fairs	2,310.98	21,891.32	21,472.01	2,730.29
Science & Society	811.82	600.00		1,411.82
Research	6,118.57	6,554.00	9,634.80	3,037.77
Natural Areas	—13.50	575.00	310.00	251.50
J. S. Wright Library	134.28			134.28
Lilly III Library	2,922.82		182.09	2,740.73
Lilly IV Library	15,000.00		3,966.75	11,033.25
Library Binding	1,033.55	1,000.00	2,033.15	0.40
Publications	997.01	8,668.39	8,168.39	1,497.01
NSF Grant	12,770.65	8,349.97	15,667.79	5,452.83
	<u>\$43,920.55</u>	<u>\$49,238.68</u>	<u>\$63,022.71</u>	<u>\$30,136.52</u>

III. SUMMARY

	Academy Accounts	Administered Accounts	Total
1971 Income	\$ 8,800.64	\$49,238.68	\$58,039.32
1971 Expenditure	11,890.62	63,022.71	74,913.33
Net Loss 1971	—3,089.98	—13,784.03	—16,874.01
1970 Balance	10,296.09	43,920.55	54,216.64
December 31, 1971 Balance	7,206.11	30,136.52	37,342.63

IV. BANK BALANCES

Terre Haute First National Bank, Terre Haute, Indiana	\$ 4,365.32
Great Western Savings & Loan, Los Angeles, California	11,602.80
First Western Savings & Loan, Las Vegas, Nevada	21,374.51
	<u>\$37,342.63</u>

REV. DAMIAN V. SCHMELZ, Treasurer

December 31, 1971

January 19, 1972

We, the undersigned, have audited the Treasurer's records for the Indiana Academy of Science for the year 1971 and have found them to be accurate and in order.

CURTIS A. DHONAN

ROBERT E. DOLPHIN

January 19, 1972

FINANCIAL REPORT

19

BUDGET FOR 1972

Anticipated Income

Dues, Initiation and Reinstatement Fees	\$ 5,800.00
Interest on Savings	1,200.00
Reprints: Net Profit on Sale to Authors	125.00
	\$ 7,125.00

Budgeted Expenditures:

Secretary	\$ 500.00
Treasurer	225.00
Office	175.00
Officer Travel Allowance, AAAS Conference Dues, etc.	180.00
President's Fund	100.00
Membership Committee	150.00
Program Committee	600.00
Proceedings: Editor's Travel and Office Expenses	500.00
Youth Activities: Chairman	50.00
Public Relations: Director	50.00
Speaker of the Year	
Honorarium	500.00
Administrative	50.00
Newsletter	150.00
CPA Fees: Tax Forms and Auditing	500.00
Transfers to Administrative Accounts	3,025.00
Publications: Publication of Proceedings	\$ 500.00
Publications: Mailing of Proceedings	150.00
Library Binding	1,000.00
Science & Society	600.00
Natural Areas	575.00
Junior Academy	200.00
	\$ 6,755.00

Approved by the Budget Committee at the meeting held at the Indiana State Library, Indianapolis, on December 4, 1971.

NOTES

Membership Dues—According to the Treasurer's records, the current status may be summarized as follows: 892 paid, emeritus, life, and honorary members and clubs; 105 on file from 1970 but not yet paid for 1971; 15 resigned or lost by reason of no current address; 5 deceased during this year; 103 new members (last ones to begin during 1972); 6 previous members reinstated during the year.

Our attorney has confirmed that we may increase dues during 1972 according to Phase II legislation. Accordingly: \$6.00 regular and high school club memberships; \$3.00 student membership; \$8.00 family membership; \$1.00 initiation fee for regular, club, and student; \$2.00 initiation fee for family.

Interest—The Treasurer, from the total assets of both Academy and Administered Accounts, has maintained sufficient funds in the checking account to pay current bills throughout the year; the remainder has been invested in savings certificates: Certificates redeemed during 1971. 1) \$10,000.00 invested at 5.25% February 1971; September 1971 redemption value \$10,324.81. 2) \$5,000.00 invested at 5.25% April 1971; September 1971 redemption value \$5,139.09. 3) \$5,000.00 invested at 5.75% February 1971; December 1971 redemption value \$5,185.29.

Certificates current: 1) \$10,000.00 invested at 6.00% April 1970; current value \$10,941.86; maturity April 1972. 2) \$5,000.00 invested at 5.75% April 1971; current value \$5,205.39; maturity April 1972. 3) \$4,465.07 invested at 5.75% April 1970; current value \$4,938.07; maturity March 1973. 4) \$6,000.00 invested at 6.00% April 1970; current value \$6,664.73; maturity March 1973. 5) \$5,000.00 invested at 5.75% October 1971; current value \$5,061.87; maturity October 1972.

Treasurer—Expenditure exceeded budget mainly due to considerable amount of xeroxing materials and telephone calls relative to work with the attorney on tax status problems.

Program Committee—Expenditure exceeded budget due to some 1970 bills which arrived months late.

Newsletter—Expenditure exceeded budget this first year for the newsletter. Next year's budget has been increased.

Reprints—Volumes 78 & 79 appeared in 1970. Payments by authors were not completed until this year; this account showed a deficit in 1970; net profit to the Academy = \$572.54.

Volume 80 appeared this year. Currently more than one third still owe for reprints. Anticipated profit = \$125.00.

The profit to the Academy pays for office expenses in billing and the expense for President's address, necrology report, and instructions for contributors.

Attorney Fees—ICE, MILLER, DONADIO & RYAN of Indianapolis have been representing us in clarifying our tax-exemption status. One additional quarterly billing can be expected in 1972. Although IRS has not completed study of documents and has not sent formal ruling, the attorney has instructed us to continue operating under the assumption of a final favorable determination.

The 1972 budget allows for contracting a CPA firm to prepare the Indiana and Federal Non-Profit Organization Tax Forms and to perform an informal audit of the Treasurer's records.

Science Talent—Refunds of \$67.50 from the director and \$16.00 from *Scientific American* have not been entered in this report as either income or expenditure.

Science Fairs—A refund of \$2,866.32 has been entered in this report as both income and expenditure.

The unusually large expenditure is explained by \$13,500.00 having been transferred to the Indiana Science Education Fund, Inc.

Research—\$5,870.00 was transferred from the Indiana Academy of Science Invested Income Account 11-430-2 (income) to pay for *Monograph 2* (expenditure).

Other income includes \$384.00 from A.A.A.S. and \$300.00 annual transfer from the Indiana Academy of Science Foundation Fund 11-430 for research grants.

Other expenditure was \$3,764.80 in grants to nine Academy members.

NSF Grant—Request for extension on use of funds, originally scheduled for August 31, 1971, was granted first until December 31, 1971, and most recently until June 30, 1972.

Publications—Income includes \$7,565.89 transferred from the J. S. Wright Fund 11-430-1 and \$500.00 from Academy Accounts to pay for publishing Volume 80 of the Proceedings.

The State of Indiana contributes \$4,000.00 towards the publication of each volume of the *Proceedings*, provided we contract with the state-approved publisher; this amount is subtracted from our bill by the publisher; the state pays the publisher directly so that this amount never appears on the Treasurer's records.

Additional income includes: \$65.00 for sale of *Proceedings*; \$285.50 for sale of *Natural Features*; \$152.00 for sale of Monographs; \$100.00 transferred from Academy Accounts for mailing Volume 80.

Additional expenditure includes \$102.50 for mailing Volume 80.

Budget for 1972—Increase from 1971 budget: Membership Committee from \$75.00 to \$150.00. *Proceedings*: Editor from \$400.00 to \$500.00. Newsletter from \$100.00 to \$150.00. *Proceedings*: Mailing from \$100.00 to \$150.00.

New items: Youth Activities: Chairman. Public Relations: Director. CPA Fees.

Change from Academy Accounts to Administered Accounts: Junior Academy.

INDIANA JUNIOR ACADEMY OF SCIENCE

OFFICERS

President: JOHN JOSEPH FARRELL, Brebeuf Preparatory School,
Indianapolis, Indiana

Vice-President: DENNIS DEROSA, Morton High School, Hammond,
Indiana

Secretary: BARBARA KONKLE, Madison High School, Madison, Indiana

JUNIOR ACADEMY COUNCIL

HELEN REED, (1971), Manual High School, Indianapolis, Indiana

SISTER MARY ALEXANDRA, (1972), Roncalli High School, Indianapolis,
Indiana

MARY J. PETTERSEN, (1973), Morton High School, Hammond, Indiana

LAWRENCE E. POORMAN, (1974), Physics Department, Indiana State
University, Terre Haute, Indiana

STATE DIRECTOR

DONALD R. WINSLOW, Owen Hall 205, Indiana University, Bloomington,
Indiana 47401

REGISTRATION

All members, sponsors, and guests were requested to register (no
charge) in Dennis Science Hall, Lobby, 8:00 AM to 11:00 AM.

PROGRAM

Thirty-Ninth Annual Meeting

Earlham College Campus Richmond, Indiana

Saturday, October 30, 1971

8:00 AM

Junior Academy Council Meeting. Dennis Science Hall, 2nd Floor
Conference Room

8:30-10:00AM

Junior Academy Council Interviews for "Best-Boy"—"Best-Girl"
Scientists Awards. Dennis Science Hall, 2nd Floor Conference Room.

8:30-10:00AM

Tours and Demonstrations of Research Facilities, Faculty-Student
Research Projects. Arranged by ANSEL M. GOODING, Earlham
College.

10:00-11:30 AM

General Session I, JOHN FARRELL, presiding. Dennis Science Hall,
Room 110.

PROGRAM OF PAPERS

"The School Ecology Club—An Active or Passive Role", University School Ecology Club, Bloomington, GEORGE LUGINBILL, sponsor.

Invitational Papers

- 1) The Symptoms of Anxiety Neurosis that Result from Injections of Lactic Acid. KEVIN POELHUIS, Harrison High School, Evansville.
- 2) The Effect of Time on the Transfer of Information for Cell Division in *Escherichia coli* B/r. DALE PURDY, Student, Indiana University, Bloomington. (High school research funded by T. A. Kleckner Science Grant Award, Indiana Heart Association Grant)
- 3) Variance in Visual Latency on the Human Retina. MARK CRISWELL, Morton Senior High School, Hammond.
- 4) The Effects of Acetylstrofludin on Conduction and Automaticity of a Mammalian Heart. DEBORAH RAJSKI, Ladywood-St. Agnes School, Indianapolis.
- 5) Design and Construction of a Laser for Research Purposes. RAY LICHTENHAN, Morton Senior High School, Hammond.
- 6) The Effects of Adrenal Demedullation on the Urinary Catecholamine Concentration as Related to Pheochromocytoma. MILTON J. MILLER II, Harrison High School, Evansville.

11:30 AM-1:00 PM Lunch

1:30 PM

General Session II, JOHN FARRELL, presiding. Dennis Science Hall, Room 110.

ANNUAL BUSINESS MEETING

Election of Officers

Address: "Synthesis from Divergence: The Coming Revolution in Science" LAWRENCE E. POORMAN, Associate Professor of Physics, Indiana State University, Terre Haute.

3:00 PM

General Announcements, Presentation of Awards, Adjournment. DON WINSLOW, State Director.

AWARDS

Awards will be presented to the "Best Boy" and "Best Girl" scientists by the American Association for the Advancement of Science. Nominations may be sent by teachers to the Director (October 22 deadline) at the following address:

DONALD R. WINSLOW, Director
Indiana Junior Academy of Science
Owen Hall 205
Indiana University
Bloomington, Indiana 47401

MINUTES OF THE THIRTY-NINTH ANNUAL MEETING OF THE INDIANA JUNIOR ACADEMY OF SCIENCE

The thirty-ninth annual meeting of the Indiana Junior Academy of Science was held Saturday, October 30, 1971, on the campus of Earlham College in Richmond, Indiana. One hundred twenty-two members representing 14 high school clubs attended.

President John Farrell called to order the morning session at 10:00 AM in Room 110 of Dennis Science Hall. Prior to the meeting members and their sponsors were given tours and demonstrations of research facilities and faculty-student research projects. These were arranged by Ansel M. Gooding, Earlham College.

A panel discussion on "The School Ecology Club—An Active or Passive Role" was presented by members of The University School Ecology Club. Mr. George Luginbill, sponsor, moderated the presentation.

Vice-President Dennis DeRosa presided over the presentation of papers. Those presenting papers were Kevin Poelhuis, Milton J. Miller II, Harrison High School, Evansville; Dale Purdy, Indiana University, Bloomington; Mark Criswell, Ray Lichtenhan, Morton Senior High School, Hammond; and Deborah Rajske, Ladywood-St. Agnes School, Indianapolis.

The afternoon session was called to order at 1:30 PM by President John Farrell. The election of officers followed; nominations were received from the floor. The results were as follows:

President—RAY LICHTENHAN, Hammond Morton, Indianapolis

Vice President—ERIC VALAINIS, Brebeuf Preparatory School

Secretary—LAURA FISHER, Lew Wallace Senior High

A presentation by Lawrence E. Poorman, Associate Professor of Physics, Indiana State University, Terre Haute, followed. It was entitled "Synthesis from Divergence: The Coming Revolution in Science."

Next, the AAAS award for the "Best Boy Scientist" was presented to Mark Criswell of Hammond Morton. The "Best Girl Scientist" award was presented to Laura Fisher, Lew Wallace Senior High School, Gary.

President John Farrell adjourned the meeting at 3:00 PM.

Respectfully submitted,
BARBARA KONKLE, Secretary



FIGURE 1. Front row: Miss Laura Fisher, Freshman, Lew Wallace High School, Gary—"best girl" and Mr. Mark Criswell, Senior, Morton Senior High School, Hammond—"best boy". Back Row: Mr. Lee E. Moss, Lew Wallace High School, Gary, sponsor of Miss Fisher; Donald R. Winslow, State Director, Indiana Junior Academy of Science, Indiana University; and Dr. Mary J. Pettersen, Hammond, sponsor of Mr. Criswell. Photo taken at Earlham College, October 30, 1971, by Richard Rodgers, Photographer, Richmond, Indiana.

NEW CLUBS For 1971

<u>Club</u>	<u>School</u>	<u>Sponsor</u>
Griffith Science Club	Griffith Senior High School	LYNN OROUTT
Science and Math Club	Lowell High School	OMER BEECHING and LAWRENCE OUWENGA
East Washington Science Club	E. Washington High School, Pekin	J. E. EVENSOLE
Physics Club	St. Joseph Academy, Tipton	SISTER MARY ANN, C.S.J.
Indian Creek	Indian Creek Senior High, Trafalgar	HAROLD HECKMAN
Beech Grove High School Science Club	Beech Grove High School	BEN CHAPIN

NECROLOGY

FAY KENOYER DAILY, Butler University

(SAMUEL) CLIFFORD ADAMS

Rockville, Indiana
February 12, 1912

Hanover, Indiana
August 13, 1971

Dr. (Samuel) Clifford Adams, a geology professor, was at his desk at Hanover College when he died August 13, 1971, of a heart attack.

Born on a farm near Rockville, Indiana, February 12, 1912, Dr. Adams was of English descent. He graduated with a Bachelor of Arts Degree *cum laude* at Hanover College in 1935; obtained a Master of Science Degree (1940) and a Doctor of Philosophy Degree (1942) from the University of Iowa. He was an instructor of geology at Hanover College from 1935 to 1937, and clerk of mapping for the Agricultural Adjustment Agency, United States Department of Agriculture in Indiana from 1936 to 1937. He was an assistant at the University of Iowa from 1938 to 1942. He also served as Junior Geologist with the Soil Conservation Service, United States Department of Agriculture from 1939 to 1941, and field assistant for the Iowa Geological Survey in 1940.

In 1942, he entered the United States Army and served until 1945. He was a combat intelligence officer and was permanently blinded by enemy mortar fire during the invasion of the Leyte Islands, the Philippines. For his heroism, he received the Combat Infantryman Medal, the Bronze Star and the Purple Heart. Posthumously, his wife received a certificate signed by President Nixon which states: "The United States honors the memory of Samuel C. Adams. This certificate is accorded by a grateful nation in recognition of devoted and selfless consecration to the service of our country."

In September, 1945, Dr. Adams returned to Indiana to teach geology as an associate professor at Hanover College. Familiar with the campus from student days, he had little difficulty finding his way around, which he accomplished unassisted. An article in the magazine section of the *Indianapolis Star* (February 2, 1947) commended his classes. With the use of models, blackboard illustrations and free discussions, his classes maintained a high degree of interest. It was stated that he was the only professor in a Hoosier College, blinded in World War II actively engaged in teaching at that time. His wife, Mrs. Blanche Adams, also taught at Hanover College. They were both alumni of Hanover and she was a student at the University of Iowa at the same time as Dr. Adams. Clifford Adams was especially interested in soil erosion, recent stream and valley sedimentation, accelerated

sedimentation of the Galena River Valley, soil conservation, stratigraphy and sedimentation, general, surficial and structural geology and ground water. He became a full professor in 1951 serving the college with great distinction.

Dr. Adams joined the Indiana Academy of Science in 1945 and was a Divisional Chairman of the Geology and Geography Section in 1953. He delivered a paper at that meeting on *Modern Floodplain Deposits*. In 1962, he gave a talk before the History of Science Section of the Indiana Academy of Science on *A Century and a Quarter of Geology at Hanover College*. The study of Hanover history was a hobby for Dr. Adams. He was also very interested in sports, particularly football and baseball.

In addition to belonging to the Indiana Academy of Science, Dr. Adams was a Fellow of the Geological Society of America and belonged to the Geochemical Society, American Geophysical Union, Iowa Academy of Science, Sigma Xi, Delta Epsilon, Pi Gamma Mu, and was a charter member of the Indiana Section of the American Institute of Professional Geologists. He was awarded an alumni achievement award from Hanover College in 1967.

Poised and sure, Dr. Clifford Adams faced a dark world with confidence and without self pity. He leaves with us the inspiration of an exemplary life.

HERBERT FAUST CALL

Madison County, Florida
October 2, 1904

Indianapolis, Indiana
September 1, 1969

Few practicing physicians have been a member of the Indiana Academy of Science, but Dr. Herbert Faust Call, a pediatrician, joined in 1935 because of a life long interest in natural science and because of his interest in archeology. These interests stemmed from early boys camp experiences and the collection of Indian relics and butterflies. He became a world traveler and a sportsman. On a "camera safari", Dr. Call and Katharine, his wife, visited eight national game preserves in East Africa in 1966. Then, they took their daughter Karen (now a pediatrician) and their son, William H. (an otolaryngologist) with them to the Fiji Islands, Australia and New Zealand in 1968.

Born in Madison County, Florida, October 2, 1904, Dr. Call came to Indiana as a boy and attended grade and high school in Anderson, Indiana. He then attended Indiana University Medical School. He took his internship there and a residency at the James Whitcomb Riley Hospital for Children, receiving an M.D. Degree and his license to practice in Indiana in 1929. Post graduate work was done at Washington University at St. Louis, Missouri, and at the Children's Memorial Hospital in Chicago, Illinois. He opened his first office in Indianapolis in 1932 and developed one of the largest practices in pediatrics in Indiana.

Dr. Call was also a member of Sigma Chi Fraternity, Phi Rho Sigma, medical fraternity, Marion County Medical Society, the American Medical Association and the Woodstock Club. A good biographical account of Dr. Call appeared in the *Indianapolis Star*, September 2, 1969.

Cancer was the cause of Dr. Call's death, September 1, 1969, bringing a busy, successful career to a close at only 65 years of age (lacking about a month).



DR. RALPH E. CLELAND
Distinguished Professor Emeritus of Botany
Indiana University

RALPH ERSKINE CLELAND

Le Claire, Iowa
October 20, 1892

Bloomington, Indiana
June 11, 1971

Dr. Ralph Erskine Cleland was a Distinguished Service Professor Emeritus at Indiana University when he suddenly died of a heart attack

in his office June 11, 1971. He had gained international recognition for his study of the heredity and evolution of the evening primrose, *Oenothera*.

Born in Le Claire, Iowa, October 20, 1892, Dr. Cleland was reared in Philadelphia. He attended the University of Philadelphia where he received an A.B. degree in 1915 with a major in the classics and history. During his junior year, a friend suggested that he take a course in botany. He was so interested that he became a botany major receiving an M.S. in 1916 and a Ph.D. in 1919. Reprints of his Ph.D. thesis on the red alga, *Nemalion*, were lost at sea. He received honorary degrees from the University of Pennsylvania, Hanover College and Indiana University. He served the United States in Italy during World War I from 1917 to 1918.

He began his teaching career as Instructor of Biology at Gaucher College, Baltimore, Maryland, in 1919, and had become chairman of the department before leaving in 1938. He was an instructor at the Woods Hole Marine Laboratory in 1924, and a Guggenheim Fellow from 1927 to 1928 when he traveled to Germany to study. He had begun research on *Oenothera* while at Gaucher and had published a joint paper with A.F. Blakeslee in 1931 on analytical procedure in the study of genetic behavior in this genus and had begun to work out the probable evolutionary pathways in speciation.

In 1938, he moved to Indiana University to become professor of botany and head of the department. In 1946, Dr. Cleland's research received further recognition and financial support as one of several participants in a 6-year program in the field of genetics financed by the Rockefeller Foundation and Indiana University. He became Dean of the Graduate School from 1950 to 1958 and Distinguished Service Professor in 1958. He retired in 1963 becoming Distinguished Service Professor Emeritus. He was co-director of the Indiana University Aerospace Research Applications Center from 1963 to 1967.

Dr. Cleland joined the Indiana Academy of Science in 1938, the year he began teaching at Indiana University and was honored as a Fellow in 1940. Even though he was active in many other organizations both national and international, he devoted considerable time to our society and contributed many papers on *Oenothera* at the sectional meetings. He was a divisional chairman of the Botany Section in 1941, president in 1959 and served on many committees. Recently, he was active in planning the establishment and program of the Science and Society Committee.

Dr. Cleland was a member of many other societies and received many honors and positions of responsibility from them. He served as editor-in-chief of the *American Journal of Botany* from 1940 to 1946 and was president of the Botanical Society of America in 1947. He was awarded a certificate of merit as a distinguished botanist by that society "for his extensive researches into the species relationships and segmental interchange problems in *Oenothera* and also for his statesmanship in representing plant science at the national level" (Golden

Jubilee Volume of the Botanical Society of America, William Steere, Ed., McGraw-Hill Co., N.Y., 1958).

He was a member of a group instrumental in forming the American Institute for Biological Sciences. While the Institute was still under the aegis of the National Academy of Science and National Research Council (1948 to 1949), he was a chairman in the society. He was chairman of the Division of Biology and Agriculture of the National Research Council from 1949 to 1952, editor of the section on plant cytology of *Biological Abstracts* 1925-. He was elected to the National Academy of Sciences in 1942; the Philosophical Society, which presented him with the Lewis Award in 1937, and he was Fellow of the American Academy. He was secretary of the Society of Naturalists from 1938 to 1940 and president in 1942. He was president of the Genetics Society in 1956 and vice-president in 1954. He was a member of the Society of Study of Evolution and was vice-president (1953 to 1958) of the International Union of Biological Science. Dr. Cleland was starred in the *American Men of Science* and was the subject of numerous articles in newspapers, scientific journals and biographical publications.

In 1960, Dr. Cleland and Dr. Farrington Daniels, vice-president of the National Academy of Science were "academic ambassadors of Science" on a visit to 11 Asian countries to meet with Asian leaders, both scientific and governmental, to discuss the possibilities of the development of these countries by science and technology. They planned to give a number of lectures to lay and professional groups on solar energy and plant genetics.

In 1948, at a Paris meeting, the Committee of Science and Social Relations of the International Council of Scientific Unions discussed science and the maintenance of peace. Questions were directed to the National Research Council Committee on UNESCO and Dr. Cleland was one of the persons replying to the request. He said (*Science and the Maintenance of Peace* by Bart J. Bok, 1949. *Science* 109:131-137): "Most modern wars have been brought about as a result of economic pressures, based on the Malthusian principle. Only science can relieve the basis of these pressures. Scientific control of population, the production of more efficient crop plants and domestic animals, the proper exploitation of vast areas of potentially usable land, control of soil erosion, development of irrigation and other projects designed to increase arable land, the discovery of new sources of energy—these are activities which will remove the chief cause of war. Without such activities, no amount of talk or education will achieve lasting peace."

While chairman of the botany department and dean of the graduate school at Indiana University, Dr. Cleland and his wife were guests at the first state dinner to which leading scientists were invited by President and Mrs. Eisenhower.

Dr. Cleland was the subject of an interesting article in *The Indianapolis Star Magazine* (May 13, 1962). After describing Dr.

Cleland's scientific achievements, the author wrote: "One gets the impression in talking with this great scientist that primroses must have a tranquilizing influence, for Professor Cleland's calm and gentle manner gives no hint of his abilities as a dynamic, efficient administrator." Then later he wrote: "Professor Cleland is a deeply religious man. He holds that there is no true conflict between science and religion. 'Both science and religion', he says, 'have the same goal; that is the finding of truth.'"

Dr. Cleland was quite active until death. One would invariably find him in attendance and often in a place of honor at national and international botanical meetings. He had just sent the manuscript to the publisher for his new book, *Oenothera Cytogenetics and Evolution*, dedicated to his wife, Elizabeth. Three sons are also scientists. They are William W. Cleland, professor of biochemistry at the University of Wisconsin, Robert Erskine Cleland, professor of botany at the University of Wisconsin and Charles F. Cleland, assistant professor of biology at Harvard University. A brother, Dr. James W. Cleland, is a Philadelphia pediatrician.

The Indiana Academy of Science lost a great friend and valuable member with the death of Dr. Ralph E. Cleland.

C (LARE) F (RANCIS) COX

Hudson, Illinois
March 9, 1888

Indianapolis, Indiana
February 24, 1971

Dr. Clare Francis Cox considered secondary education as the most stimulating professional field, because of the opportunity to encourage young people to develop an interest in science and specifically, ecology. He taught at Arsenal Technical High School in Indianapolis, Indiana, from 1917 to 1958, becoming head of the botany department in 1931. After he retired, an annual science award was established in his name in appreciation of his services.

With Milo H. Stuart, he was influential in establishing a nature preserve at Tech (part of the campus later replaced by a school) and he sponsored a nature study group there for many years. He was active in the Indiana Nature Study Club. He also taught Microbiology at the Indiana College of Mortuary Science 1958 and 1959.

Dr. Cox was born in Hudson, Illinois, March 9, 1888, and attended a country elementary school. He rode horseback to high school in Vandalia, Illinois. His teaching career began in a country elementary school the year after graduating from high school. He attributed his knowledge of birds, wildflowers and trees to his country boyhood rambles and curiosity. He also taught high school and was a basketball coach from 1913 to 1916 teaching at Herrin, Illinois, and Marion, Illinois.

He began his college education during this period, too, receiving a Bachelor of Arts Degree from Illinois State University in 1917. Immediately afterwards, he started teaching at Arsenal Technical High School and also served in the United States of America Air Force Reserve from 1917 to 1919.

A Doctor of Philosophy Degree was obtained at Chicago University in 1931. The influence of John Merle Coulter, while he was there, further stimulated his interest in science. Also, summer field trips in Utah and Colorado taught by Dr. Henry Cowles and Dr. George D. Fuller influenced Dr. Cox to study ecology and alpine vegetation. He became known here and abroad as an authority in this field. For three summers he and his wife established headquarters at 12,000 ft elevation near James Park, Colorado, in an abandoned gold miner's shack and explored the surrounding peaks for alpine plants. Tolland, 14 miles away, was their supply base to which they hiked every week climbing 3,000 feet to reach it.

Dr. Cox joined the Indiana Academy of Science in 1922 and was honored by being elected to Fellow in 1935. He served on the Junior Academy of Science Committee in 1939, 1940 and 1941. He also wrote a memorial to Emerson Blane Wright for the Necrology Report for 1928 (*Proceedings*, V. 38, 1929).

Dr. Cox was also a member of the American Association for the Advancement of Science, Ecological Society of America and was elected to Sigma Xi at Chicago University where he held a Coulter Fellowship for one year. He was life deacon at the Woodruff Place Baptist Church. Dr. Cox is listed in *American Men of Science* and *Indiana Scientists*. He has a son, Dr. Milton D. Cox, teaching at Miami University.

Dr. Clare F. Cox died February 24, 1971, at 82 years of age. He had lived a full life, but considered his personal influence on his pupils and their enthusiastic response to his teaching as his greatest satisfaction. This is the ultimate reward for the dedicated teacher.

G(EORG) K(ARL) NEUMANN

Hamburg, Germany
June 6, 1907

Bloomington, Indiana
April 13, 1971

The practical application of many academic scientific research activities is obscure, but this was not so with the work of Dr. Georg K. Neumann. Widely known for his knowledge of the physical characteristics of human skeletons, Dr. Neumann was a consultant to the Indiana and Illinois state police departments in identification of unknown persons. In an *Indianapolis News* story (April 2, 1952) some of the more famous murder trials in which he had testified were discussed. It was astonishing that a bit of bone, even a charred bone, could reveal the sex, age, size and region of ancestry of the deceased. Dr.

Neumann was an associate professor of anthropology at Indiana University and an archeologist. He had written two books and a book chapter relative to these subjects. He had participated in many Indian site excavations and was an authority on American Indians, their physical types, racial differentials and racial history.

Born in Hamburg, Germany, June 6, 1907, Dr. Neumann received his higher education in America obtaining a Ph.B. degree in 1930, an A.M. in 1936 and a Ph.D. in 1943 from the University of Chicago. He taught at the University of Chicago, Western Reserve University and was assistant director of the University of Tennessee archeological projects. He left the museum of anthropology at the University of Michigan to become an assistant professor of anthropology at Indiana University in 1942.

Dr. Neumann also became a member of the Indiana Academy of Science that same year, 1942, and was honored as a Fellow in 1957. He was a popular chairman of the Anthropology Section having served in 1943, 1944, 1949, 1956 and 1959. He was to have served again in 1971. He presented numerous papers at Academy meetings concerning Indian physical types, racial affiliations, migrations and culture. He had served a number of years on the Fellows Committee and was a member at his death. Dr. Neumann also belonged to the Central States Anthropological Association, of which he had been president; to Phi Beta Kappa, honor society; and to the Mexican and German Societies of Anthropology.

Not only were the macabre details of the murder trials in which Dr. Neumann testified newsworthy to reporters, but also the mysteries of aboriginal life determined by Dr. Neumann from the bones excavated from Indian mounds. Thus, several articles about his work in this field appeared also in Indianapolis newspapers. Some of these appeared in the *Indianapolis Times* (December 26, 1948), the *Indianapolis Star* (August 4, 1956) and *Indianapolis News* (April 2, 1952), and upon moving to Indiana University, his professional qualifications were discussed in an article in the *Indianapolis Star*, February 7, 1942.

Dr. Georg Karl Neumann died April 13, 1971, at only 63 years of age, when his unusual talent was lost to us prematurely.

CLAUDE E (DGAR) O'NEAL

Coatesville, Indiana
January 29, 1884

Delaware, Ohio
February 8, 1971

Dr. Claude Edgar O'Neal, botanist, died February 8, 1971. He had been a member of the Indiana Academy of Science since 1912, joining when a student working on a graduate degree at Indiana University. He gave a paper in 1914 on the species of *Nummularia* common in Indiana. Later, he became interested in the cytology of *Datura* and the embryologic development in *Oenothera*.

Dr. O'Neal was a native of Coatesville, Indiana, born January 29, 1884. He attended Indiana State Teacher's College in Terre Haute for 4 years where he received a certificate. He then attended Indiana University where he received an A.B. in 1911, was a teaching fellow 1912 to 1913, obtained an A.M. in 1913 and a Ph.D. in botany in 1922. He was head of the Department of Science at Brazil (Indiana) High School; principal of the Coatesville (Indiana) High School and Hadley (Indiana) High School; and taught at Reno, Indiana. He became an instructor at Ohio Wesleyan University in 1913 and served as head of the botany program until retirement. In 1920, he was named Professor of Botany on the Allen Trimble Foundation. He taught there until his retirement in 1954 when he became Professor Emeritus. He also taught one term after that from 1957 to 1958. In recognition of his service to Ohio Wesleyan University, the greenhouse adjacent to Bigelow-Rice Science Hall was dedicated as the Claude E. O'Neal Greenhouse in 1964. Former students and colleagues joined in this tribute. A memorial statement said, "The building housing the beauty and vitality of growing plants and, in its utilitarian function, serving his driving interest in botanical education, shall always stand as a fitting memorial."

Besides his membership in the Indiana Academy of Science, Dr. O'Neal was a member of Phi Beta Kappa, Sigma Xi and Omicron Delta Kappa, honor societies. He had been vice-president of the Botanical Section of the Ohio Academy of Science twice, and president of the Ohio Academy of Science from 1938 to 1939. He also belonged to the American Association for the Advancement of Science and Botanical Society of America.

Although Dr. O'Neal had been in Ohio for many years, he kept in touch with former teachers, students and friends in Indiana. The high regard which they held for him was quite evident. His former students are too numerous to be mentioned, but hold many positions of honor and distinction as professors, department chairman, directors of research or heads of national botanical laboratories. Letters of love and gratitude from them to Claude and Mabel O'Neal were a great satisfaction.

MILO BOSTWICK SAMPSON

Erie, North Dakota
June 18, 1908

Bloomington, Indiana
April 10, 1971

Dr. Milo B. Sampson was a professor of physics at Indiana University and a leading authority on cyclotron design and operation. He was in charge of the development of a large cyclotron at Indiana University when he died, April 10, 1971, at his home. He had received a grant from the National Science Foundation for this work.

Born at Erie, North Dakota, June 18, 1908, Dr. Sampson went to the State College of Washington, where he received a Bachelor of

Science Degree in 1931 and a Master of Science Degree in 1933. He was a Queen scholar and obtained a Doctor of Philosophy degree in physics at Princeton in 1937.

Dr. Sampson was a physicist at the Biochemical Research Foundation of the Franklin Institute, Philadelphia, from 1937 to 1942. From 1942 to 1943, he was research associate in the Office of Scientific Research and Development and the National Defense Research Committee at Princeton. From 1943 to 1944, he was physicist and section chief of the metallurgical laboratory, University of Chicago Laboratory, where the first nuclear reaction had been achieved in 1942. In 1944 to 1946, he was physicist at the Los Alamos Science Laboratory in New Mexico. In the atomic energy laboratories there, the atomic bomb was made. It was tested July 16, 1946, in the desert region just west of Sierra Oscura, about 50 miles northwest of Alamogordo.

Dr. Sampson came to Indiana in 1946 as an assistant professor at Indiana University. He became associate professor in 1952 and professor in 1958. He was in charge of cyclotron research from 1946 on. The old cyclotron at Indiana University, which has been dismantled, was one of the first to be built in the country.

Dr. Sampson joined the Indiana Academy of Science in 1967, and was author or co-author of a number of papers presented at sectional meetings before and after that date. These dealt, primarily, with the results from use of the cyclotron and the measurement of energy levels with a spectrometer. He was also a member of the American Physical Society, a member of Sigma Xi, scientific honorary society, Phi Kappa Phi and Pi Mu Epsilon. Biographical sketches can be found in *American Men of Science*, *Indiana Scientists*, and the *Indiana University Alumni Magazine* (1971, V. 33, No. 9, p. 40).

Awesome nuclear power under the control of man, although devastating and controversial as an instrument of war, has undoubtedly saved more lives than it has taken because of these qualities. It is thus a deterrent to war between nations possessing it. Furthermore, with wise management, it is sure to find a prominent place as a peacetime power source. Radio isotopes have improved man's lot remarkably in the field of medicine. These accomplishments will stand as a tribute to physicists like Milo B. Sampson who were intelligent and resourceful enough to seek the truth about nuclear energy and how to control it.

NEW MEMBERS FOR 1971

The following list contains the names and addresses of all new members who joined during 1971. The letter(s) following the address indicates the Section of the Academy in which the member has indicated his major interest, according to the following code:

A—Anthropology	N—Engineering
B—Botany	O—Cell Biology
C—Chemistry	P—Physics
D—Science Education	R—Microbiology & Molecular Biology
E—Entomology	S—Soil Science
G—Geology and Geography	T—Plant Taxonomy
H—History of Science	Y—Psychology
L—Ecology	Z—Zoology
M—Mathematics	

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PART 2

ADDRESSES
AND
CONTRIBUTED
PAPERS

Richmond, Indiana
October 29, 1971

PRESIDENTIAL ADDRESS

The address, "Minicourses and the Audio-Tutorial System," was presented by retiring president, Dr. Samuel N. Postlethwait, Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, at the annual Fall Meeting dinner at the Runyan Center, Earlham College, Richmond, Indiana, on Friday, October 29, 1971.

SPEAKER-OF-THE-YEAR ADDRESS

The address, "The Role of Scientists in the Preparation and Evaluation of Environmental Impact Statements", was presented by the Speaker of the Year for 1971-72, Dr. Alton A. Lindsey, Professor of Plant Ecology, Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, at the annual Fall Meeting luncheon at the Runyan Center, Earlham College, Richmond, Indiana, on Friday, October 29, 1971.

PRESIDENTIAL ADDRESS

Minicourses and the Audio-Tutorial System

S. N. POSTLETHWAIT

Department of Biological Sciences
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A student can select four variations of coffee from an ordinary vending machine, but when he enters the classroom he may receive instruction identical to that of several hundred other students. It is an obvious fact of life that people exhibit great diversity, interests and capacities, yet our educational system is made up of large blocks of content (courses) with little or no provision to break the lock-step of time, content or instructional procedure. There is some justification for the classroom approach however, since education is not just a dispensing procedure and the teacher is vital to the learning process. Many people can trace their excitement about a specific subject to the special way the subject was presented by a great teacher. Unfortunately these "great teachers" are rather rare and physical limitations permit only a few students to "sit at their feet." While it is true that the talent of these teachers has been made available to some degree through their writings, many good teachers never write for publication, and even so, the limitation imposed by the printed word negates the potential of the teacher for the clever use of tangible objects and sound.

Hopkins has suggested that the best learning situation is the "teacher on one end of the log and the student on the other." Hopkins assumed the teacher was a "good" teacher and, if one can assume the log was figurative, the concept serves as a proper model for the Audio-Tutorial (AT) system. The AT system used in conjunction with minicourses, while retaining compatibility with the conventional educational system, has great potential for providing students with improved access to "good" teachers and with some other important features such as greater individualization. This paper attempts to describe a pilot study currently being conducted at Purdue University.

History of the AT System

At Purdue University the author began an attempt to provide a remedial program in a freshman botany course in 1961. The initial effort involved the production of a weekly lecture on audio tape. This was soon expanded to include: 1) tangible objects (specimens, experimental equipment, models); 2) printed materials (texts, study guides, journal articles); and 3) projected images (slides and movies). Ultimately the program was produced by assembling the appropriate items ("logs") and, while sitting among these items, recording on audio tape the conversation one would expect to use with one student while tutoring the student through a sequence of learning activities. The product, *i.e.* the tape and

other materials, was then duplicated as many times as necessary to accommodate all students. Because of student enthusiasm for the program the procedure was expanded to cover the content for the entire botany course. The course was then restructured to include three major study sessions:

1) Independent Study Session (ISS)

The audio tutorial program was placed in a learning center which was open from 7:30 AM to 10:30 PM Monday through Friday. The student could come in at his convenience and check into a booth. On his way to the booth he would pick up a mimeographed sheet of objectives written in behavioral terms. Other components needed to complete the program (except for the student's own copy of the textbook and study guide) were housed in the booth. Materials too bulky or too expensive to include in each booth were placed on a central table for common use by all students. The student placed the headphones in position and by listening to the tape, he experienced a simulated one-to-one tutoring by the instructor. The student could pace his study as he desired, stopping at any point in the program to use additional resources such as supplemental texts and discussions with the instructor on duty or with peers. Each student proceeded independently of other students and was free to omit any part of the study unnecessary to help him achieve the stated objectives for the week. The prep room was equipped with a table, chairs and a coffee urn to encourage students to take frequent breaks and to enter into discussions with peers over a cup of coffee. When the student was satisfied with his progress he was free to leave without regard to scheduled class time.

2) General Assembly Session (GAS)

This session was scheduled on a weekly basis and included 300 or more students. Activities in this assembly involved an occasional lecture, special films, major exams and other activities that could be done most effectively in a large group. Attendance was required only for certain special events.

3) Integrated Quiz Session (IQS)

This session involved eight students and an instructor scheduled to meet weekly for one-half hour. The primary purpose of the session was to exploit the principle that "one really learns a subject when one is required to teach it". For this session each student was expected to prepare a little lecture about each of the items (logs) used in the ISS. The instructor presented the items in the sequence programmed earlier and selected the student to lecture on a random basis. Thus all students were forced to organize the subject matter in their own minds and could not rely on superficial responses to instructor questions. In addition, this session provided:

- 1) direct feedback on the effectiveness of the components of the AT program;
- 2) an opportunity for each student to know at least one instructor very well;
- 3) each student to be well known by at least one instructor; and
- 4) an opportunity to take care of certain administrative details.

The AT system in this form has been adopted in a great many schools and in a broad spectrum of disciplines (one-third of the papers presented at the Second Annual Audio-Tutorial Conference held at Purdue in November 1970 dealt with subject matter areas outside the field of biology and several were concerned with programs below the college level). Obviously, its effectiveness corresponds directly to the ability of the "good" teacher to prepare the AT programs and to originate the necessary supplementary sessions. Many teachers report highly successful systems (1, 2, 3).

Minicourses and Mastery

In 1969 when Dr. Robert N. Hurst joined the staff at Purdue University with the assignment to convert a zoology course (Biology 109) to the Audio-Tutorial approach, it was decided to reorganize the content of both the zoology and botany courses into smaller units of information called minicourses. Each minicourse would cover a reasonably coherent segment of subject matter (topic) and each minicourse would have a written set of specific objects suitable for testing the student's mastery of the concepts included. No rigid guide lines were established as to length or teaching strategy. Primarily, the limits of a minicourse were determined by good judgment much as one decides on how to divide a book into chapters. Approximately 30 minicourses were identified for each course and several of these minicourses covered subjects common to both zoology and botany. It was clear that if students were required to master the objectives in their first study of the common minicourses it would be redundant to involve them with the same subject matter a second time in the subsequent course.

As each student mastered the objectives for a particular common minicourse, it was recorded on his individual card and he was not required to repeat the minicourse study again. Thus, students entering the two course sequence through either botany or zoology and taking the common minicourse during their first enrollment accumulated some time which could be devoted to the exploration of their own interests through the study of optional minicourses during the second semester of their enrollment. The botany and zoology courses which had previously been two distinct four credit hour courses now became a "pool" of minicourses divided into four categories, *i.e.*,

- | | |
|-----------------------|-------------------------|
| 1) Plant minicourses | 3) Common minicourses |
| 2) Animal minicourses | 4) Optional minicourses |

Students entering the botany-zoology complex by the way of botany were required to take the plant minicourses and common minicourses and those students entering the complex by way of zoology were required to take the animal minicourses and common minicourses. Optional minicourses were selected by the student during the second semester of enrollment as needed to complete the requirement for the full four credit hours in botany and four credit hours in zoology.

Most of the objectives for each minicourse were written at the knowledge and comprehension level, and when a student achieved mastery for a given minicourse he had a C entered on his record for that minicourse. After completing the required number of minicourses at this level, the student was awarded a grade of C in the course for which he was enrolled. A student who had not completed the number of minicourses required for the course for which he was enrolled was given a grade of incomplete with the opportunity to complete the uncompleted minicourses during the subsequent semester. At that time, the incomplete would be removed and a permanent grade would be assigned by the Registrar's office.

Students who wished to earn grades of A or B could do so by completing additional activities requiring a greater knowledge and "understanding" of science, a greater time input and more creative ability. The A-B activities were administered on a point basis. Students receiving an A were expected to acquire 110 points, while those working for a B had to acquire 85 points. These points were earned by participating in a variety of activities including special examinations, outside readings, research projects, peer tutoring activities, library projects, and other ways agreed upon between the student and instructor.

After two years experience it is clear that it is possible to combine the Audio-Tutorial system with the concepts of minicourses and mastery to develop a learning system which provides a great deal more individualization and flexibility than the conventional lecture-laboratory approach.

Some advantages and disadvantages

A partial list of advantages is as follows:

- 1) The primary learning program (AT) can be prepared by a "good" teacher and all his skill in selecting and sequencing learning activities can be made available to each student on a simulated tutorial basis.
- 2) The rate and emphasis of study is directly under the student's control. He can stop at any point in the program to obtain outside assistance (instructor, peer, book or other resources) and can repeat or skip any segment of the program appropriate to his needs.
- 3) The system is designed for success. The expectation is that all students will achieve the objectives of each minicourse. The relatively small units of subject matter are less forboding than

a complete course and a sense of accomplishment is achieved with the mastery of each minicourse.

- 4) There is great flexibility for individualizing course content to the specific needs of students. Minicourses can be selected and combined in a variety of ways to accommodate major goals, interests, capacities and backgrounds.
- 5) Redundancy can be reduced and efficient use of facilities, staff and student time can be achieved.
- 6) Selection of media and instructional strategy is limited only by the creativity of the instructor and his facilities. The nature of the subject matter and objectives dictate the procedure and materials to be used.
- 7) Programs that only involve portable tangible items could be made available outside the Learning Center. Perhaps several minicourses constituting a relatively high proportion of a regular course could be studied at home and thus reduce the cost to both the student and the school.
- 8) The transfer of materials between courses and between schools could be accomplished more readily since each minicourse is essentially an independent learning system and could be easily combined with others to adapt to the local situation.

A partial list of disadvantages is as follows:

- 1) The development and testing of an AT minicourse program is time consuming and requires considerable skill and talent.
- 2) The system requires some psychological adjustment for both student and teacher. The student must assume a greater degree of responsibility for his own progress and make some decisions for himself. The teacher must become committed to "helping students learn" and accept less focus on himself and his role in the learning process. The teacher further must adjust to having all his efforts and objectives exposed to students and colleagues for review and criticism.
- 3) Many factors only tangentially related to the system may be frustrating and create unexpected difficulties which have undue influence on the success of the program. A change from the routine within routine surroundings is never easy.

Summary

Although the pilot study using the AT system with minicourses and mastery concepts has been underway only two years, it is apparent that it is feasible and practical even in a conventional university setting. Its potential for individualization is well received by students, and the admin-

istration of the program can be accomplished by re-deploying about the same number of staff as required for conventional teaching.

The author's hopes for the botany and zoology students have nearly all been realized. Perhaps more important, however, is the potential the minicourse concept has for providing new ways of "going to college" and for assisting with equal opportunity education and continuing education. Alternatives to the existing educational system are necessary to accommodate the needs of a substantial portion of our population. Many of these people are unable to fit into the regime of "going to school" for a variety of reasons, but they still have a need and desire of expanding their education. Self-instructional minicourses in the format described in this paper can provide the necessary flexibility and portability with no compromise in the quality of instruction. The compatibility of the system with conventional procedures makes it feasible to intermix the two approaches with no loss or problem to the student. The major task now is to produce the quantity and quality minicourses necessary to accommodate the current needs and to devise the additional administrative procedures required to make such programs available on a broad scale.

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2. MELECA, C. B. 1970. The use of multivariate analysis in the evaluation of audio-tutorial programs I and II. *BioScience* 20:23-30.
3. SPARKS, P. D., and L. M. UNBEHAUN. 1971. Achievement of audio-tutorial and conventional biology students: A comparative study. *BioScience* 21:574-576.

The Role of Scientists in the Preparation and Evaluation of Environmental Impact Statements¹

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Pressure from private citizens including many environmental scientists has prompted Congress, the Administration and the Courts to improve our national policies toward the environment during 1969-1971. The Congressional Research Service identified as "environment oriented" 121 of the 695 bills signed into law during the 91st Congress. Signing of the National Environmental Policy Act (NEPA) on January 1, 1970, was President Nixon's first official action in the "environmental decade of the '70's." The Act set up a 3-man Council on Environmental Quality (CEQ) with a small staff. It deals with environment broadly, whereas the Environmental Protection Agency (EPA), born on December 2, 1970, is concerned only with air and water pollution, environmental impact of pesticides, solid waste and radiation hazards in the general environment. As an operating agency, EPA received nearly \$2.5 billion for F.Y. 1972.

NEPA requires for major federal projects that the lead agency prepare a "draft" environmental impact statement, stating the effect the proposed action would have on the environment, including unavoidable adverse effects, possible alternatives, the long-term as well as immediate impact and any irreversible and irretrievable commitments of resources. The draft statement must be available to the public for 90 days before action is taken. Public hearings are to be provided "when appropriate" which presumably means in controversial cases. Draft statements are reviewed in the 10 regional offices of EPA, and by other involved federal agencies. The public (including outside scientists) is invited to make significant inputs into revision of draft statements, and thus into federal decision-making. This concedes that the public has some expertise as to what is in the public interest. As a panel of 3 federal judges ruled, "The very purpose of NEPA was to tell federal agencies that environmental protection is as much a part of their responsibility as is protection and promotion of the industries they regulate".

¹ The Indiana Academy of Science established in 1971 a Science Communication Award of \$500., funded through the Science and Society Committee from its National Science Foundation grant. The award is presented at the general session of the fall meeting, when the recipient is called upon to give an address. He is also scheduled for visits to Indiana college campuses, to bring somewhat similar material to students, faculties and townspeople. Printed here is Dr. Lindsey's brief summary of his address given at the Earlham College meeting of the Academy on October 29, 1971. A more popularized version entitled "People, science and federal environmental policy" was given later at 5 colleges.

² The speaker wishes it recorded that the views expressed herein are his own; he is not speaking as a representative of Purdue University, the Indiana Academy of Science or any other institution.

States should license the ecological consulting firms which are springing up, so as to insure at least minimum qualifications. So far, these firms have had little business involving impact statements. There is much scientific and technical content in the areas under CEQ jurisdiction: air quality, weather modification, energy, hazardous substances including pesticides and herbicides, land use and management, noise, physiological health and human well-being (including food purity and waste systems), transportation, urban environments, water and wildlife. The Washington office of CEQ evaluates the final statements, chiefly by attorneys with some *ad hoc* reference to scientists. The Chicago regional office of EPA, one of the best, employs many technical experts, but has twice as many people trained in law and social science as in chemistry and chemical engineering. (Although EPA is often thought of as an enforcement agency, it is not empowered to take cases to court, but recommends action by prosecutors up to the Department of Justice.) A maxim in Washington says that scientists should not be on top, only on tap. But we think the tap should be turned on more often. This would make it less necessary for scientists' groups like Environmental Defense Fund to engage in litigation. Students being trained in the new environmental law specialty should take more courses in science.

The federal projects that have been pushed through without compliance with NEPA have mostly been military procurement projects. Several large public works projects have been postponed by citizen action in federal courts for lack of environmental impact statements. However, no projects have yet been finally vetoed or terminated, without court action by private groups, because an impact statement projected environmental harm. Nationally, about 1,800 impact statements have been received, for Indiana only four. Apparently, only one public hearing on an impact statement has been held in the nation. Scientists Institute for Public Information is asking the courts to require AEC to prepare, for comment and discussion of alternatives, a detailed statement on the environmental effects of, not one reactor at a time, but a nation full of liquid metal fast breeder reactors.

Since even the best impact statements prepared by the promoting agency are slanted toward advocacy, such statements for potentially controversial projects, at least, should be done by multidisciplinary teams independent of the agency, and using a broad systems analysis approach.

The provisions of NEPA have greatly enlarged the opportunity of scientists, as well as the so-called ordinary citizen, to participate directly in the operative processes of government. For this unprecedented and superb mechanism of environmental democracy to start operating as Congress intended, it will require more understanding and activism by outside scientists and other private citizens.

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ANTHROPOLOGY

Chairman: WILLIAM R. ADAMS, Department of Anthropology,
Indiana University, Bloomington 47401

EDWARD DOLAN, Department of Anthropology,
DePauw University, Greencastle, Indiana 46135, was elected
Chairman for 1972

ABSTRACTS

A Brief Synthesis of Indiana Prehistory. B. K. SWARTZ, JR., Department of Anthropology, Ball State University, Muncie, Indiana 47306.—Indiana was subdivided into three physiographic zones, Moraine and Lake, Tipton Till Plain, and Valley and Upland, and its prehistory was examined on this backdrop through six developmental stages, Lithic (usually termed Paleo-Indian in North American archaeology), Archaic, "Intermediate" (a provisional stage specifically formulated from an Indiana point of view), Woodland, Mississippian, and Woodland-Mississippian (a fusion of the two preceding, rather than a stage in itself.)

A Second Report on Earthwork Seven, the New Castle Site, a Portion of the Southeast Quadrant—1971. GARY M. HEATHCOTE, YASUO TOYODA, and B. K. SWARTZ, JR., Department of Anthropology, Ball State University, Muncie, Indiana 47306.—Earthwork 7, Hn-1 (IAS-BSU), is a small "sacred enclosure" located on the southern periphery of the New Castle Site complex. Descriptively, the earthwork is a low-lying natural knoll, measuring 67 feet by 81 feet, surrounded by an aboriginally excavated interior ditch. Excavations in the southeast quadrant this year revealed the presence of plain Woodland Ware sherds within the disturbed fill strata of the interior ditch. No diagnostic features, such as post molds, were discovered, and only one lithic artifact, a pecked stone ball, was found *in situ* within the earthwork.

Continued Excavation of Earthwork Four, New Castle Site, Indiana. HAROLD G. STACY and B. K. SWARTZ, JR., Department of Anthropology, Ball State University, Muncie, Indiana 47306.—For the past 6 years excavation has been carried on a panduriform mound known as Earthwork Four. This year the major portion of the northeast quadrant of the east mound was excavated. A total of 15 features were uncovered in the 39 5x5 foot excavation units excavated during the 1971 Summer Field School. These included three human cremations, one probable post hole, one unique soil formation, a bird burial, four ash and charcoal lenses, one group of rocks surrounding a piece of red ocher, two fire hearths (one with post holes), and two features of fire cracked rocks. During the excavation six scrapers, five pieces of nondescript pottery, two cores, three points, and a post hole filler from Feature 2 were unearthed. A rusted piece of metal and a square nail were found 48 inches below the surface in units N1-E3 and N2-E3, and another piece of rusted metal was found at the base of the mound in unit S1-E3. Also in unit S1-E3 the unexcavated portion of a cremation was uncovered. A portion of this earthwork had been excavated, possibly Redding's work in 1890.

Aerial Photography in Archaeological Survey. ROBERT E. PACE, Department of Anthropology, Indiana State University, Terre Haute, Indiana 47809. —Where extensive areas are to be searched for archaeological sites, carefully planned and executed aerial photography lends invaluable assistance. While aerial film will record a few obvious sites, its greatest value lies in identifying relationships of surface natural features, land-use patterns, and back-road networks. With this information and topographic maps, surface survey can proceed more rapidly and efficiently. At specific sites, aerial film may record evidence of archaeological features not apparent to the surface observer.

Archeology of Tell Hesban, Jordan. ROBERT LITTLE, Department of Anthropology, Indiana University, Bloomington 47401. —Tell Hesban, or ancient Heshbon, is a city mound at the edge of the plain of Moab in the Hashemite Kingdom of Jordan. It has an elevation of 895 meters above sea level and is 26 road kilometers southwest of the capital city of Amman. It is 30 kilometers east of the north end of the Dead Sea.

The first time it is mentioned in history, it was a Moabite city, then a capital city of the Amorites of the Late Bronze Age.

It was continuously occupied from that point by Israelies, Greeks, Romans, Christians, and Arabs. It was last mentioned by Arab writers in the 14 century A.D.

The first expedition to Heshbon, sponsored by American Schools of Oriental Research and staffed by 45 technicians and 165 native workers was scheduled in 1967 but had to be canceled. There was 8 successful weeks in 1968, then canceled again in 1970, and another successful 8 weeks in 1971.

Much material has been recovered and analyzed, but since the second season has only reached Iron III and possibly II and in only some of the areas, it is too early for final conclusions. About 5 more seasons will be needed to develop a clear picture of 4,000 or more years of continuous history at one location.

The Commissary Site: A Woodland Cemetery. GLORY K. HOUCK and B. K. SWARTZ, JR., Department of Anthropology, Ball State University, Muncie, Indiana 47306. —Concentrated effort was made to discover a burial left in 1968 that would allow the reconstruction of the grid system established in 1966. Stakes from the original grid and a burial corresponding to the 1968 provenience were exposed; but, the missing lower limbs of the burial and its closeness to the edge of the site cause doubt that it is the 1968 burial.

A total of 13 individuals in 9 designated burials were exposed and removed. Five individuals were removed from one unit alone. The possible 1968 burial was associated with a great number of artifacts, including beaver incisors, worked chert objects, bear canines, antler tines, a tortoise shell rattle, and the remains of what may have been a "medicine bag." In

association with another individual in the same excavation unit were an expanded stem elbow pipe of steatite and two shouldered knives.

The Henry County district soil scientist identified the dark brown soil surrounding burials as gravelly silt loam, identical in texture to the soil found in the valleys of the Big Blue and Little Blue Rivers. In his opinion, the normal subsoil of the site had been removed and replaced with the soil transported from the valley beds.

Special emphasis was placed on stratigraphy and outlining of burial pits.

Bone Growth in Two Prehistoric Indian Populations

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Abstract

The diameter of the adult radius was compared in two prehistoric Indian populations which differed markedly in diet. The Indian Knoll population of the Archaic Period was a hunting and gathering group. The Pete Klunk Mound Group population of the Middle Woodland or Hopewell Period supplemented its hunting and gathering with part-time agriculture. Therefore, the diet of the Hopewell population was larger and more varied than that of the Indian Knoll population. Statistically significant differences in radius diameter were shown to exist both inter- and intrapopulationally. Males of both populations were larger than females. Males and females of the Hopewell group were larger, respectively, than males and females of the Indian Knoll group. These population differences in bone diameter are attributed to differences in the aboriginal diets.

The effect of nutrition on circumferential growth of the radius bone was examined in two prehistoric Indian populations, the Indian Knoll of Kentucky and the Pete Klunk-Hopewell of Illinois. The Indian Knoll population (dated between 2500 and 2000 B.C.) represents the Archaic Period of Eastern United States prehistory. The subsistence activities of this group were centered exclusively around hunting and gathering in the vicinity of the Green River. The Hopewell, a more recent population (dated between 50 B.C. and 250 A.D.) represents the Middle Woodland Period. This group also relied on hunting and gathering but supplemented these activities with part-time agriculture in the Illinois River Valley. The hypothesis tested is that due to agriculture and, as a consequence, a larger and more varied diet, the amount of bone growth in the Hopewell population would be greater than that of the Indian Knoll population.

Materials and Methods

Only well-preserved, intact radii from adult males and females were studied. The sampled radii represented individuals ranging in age from 20 to 70 years. The sample was made up of 121 males and 54 females from Indian Knoll and 45 males and 46 females from Hopewell. Those radii which evidenced obvious pathology such as periostitis or arthritis were not used. The width or diameter of each radius was measured at two sites: a cortical or compact bone site at a point one third the shaft length as measured from the styloid process and a trabecular or spongy bone site, at a point one tenth the distance from the styloid process. A photon absorptiometric technique developed by Cameron (1) was employed to measure both width and bone mineral content of each radius. A monoenergetic photon beam from a low energy radionuclide, iodine-125, was passed across the width of the bone. Changes of transmittance, which are directly proportional to the bone mineral mass in the scan path, were measured with a scintillation detector-pulse height analyzer system (3). Data from each scan or measurement were transferred to paper tape by means of an eight channel Tally Paper Tape Punch. An IBM Tape to Card Converter trans-

TABLE 1. *Mean widths of radial diaphysis (cortical) and distal radius (trabecular). Width values in millimeters.*

Site	Indian Knoll			Hopewell			Difference	
	N	Width	Standard Deviation	Coefficient of Variation	N	Width		Standard Deviation
Males								
Cortical	121	13.3	1.57	11.8	45	14.4	1.38	9.6
Trabecular	121	22.5	1.92	8.5	45	23.8	2.26	9.5
Females								
Cortical	54	11.2	1.23	10.9	46	13.1	1.50	11.4
Trabecular	54	20.1	1.63	8.1	46	21.5	1.77	8.2
							1.1	(7.6%)
							1.3	(5.5%)
							1.9	(14.5%)
							1.4	(6.5%)

ferred the data to the standard 80-column cards. A computer program for calculation of radius width was written by Dr. David M. Smith of the Indiana University Medical Center and was made available for this study.

Results

Table 1 presents data on the width of the radii used in this study. In all cases the average width of the Hopewell radius exceeds that of the Indian Knoll radius. This relationship is especially evident in the 14.5%

TABLE 2. *Comparison of mean cortical and trabecular widths by sex and culture using Student's t. Indian Knoll (IK) and Hopewell (H).*

Site	Comparison	Value	Significance
Cortical	IK Males vs. H. Males	4.19	p<0.001**
	IK Females vs. H. Females	6.96	p<0.001**
Trabecular	IK Males vs. H. Males	3.74	p<0.001**
	IK Females vs. H. Females	4.12	p<0.001**
Cortical	IK Males vs. IK Females	8.71	p<0.001**
	H. Males vs. H. Females	4.32	p<0.001**
Trabecular	IK Males vs. IK Females	8.00	p<0.001**
	H. Males vs. H. Females	3.45	p<0.001**
Cortical	IK Males vs. H. Females	0.75	p>0.20
Trabecular	IK Males vs. H. Females	3.09	0.001<p<0.01*

*Significant

**Highly Significant

difference in average cortical width between the two female samples. In Table 2, Student's t-tests show that when Indian Knoll males are compared to Hopewell males or when Indian Knoll females are compared to Hopewell females, the differences in average radius width are very highly significant at the 0.001 level of confidence. Size differences are further indicated when the Hopewell females are compared to Indian Knoll males. No statistically significant difference between their respective cortical widths could be found despite a statistically significant sexual dimorphism within each population with males being larger than females (Table 2).

Discussion

In the earlier stated hypothesis, bone growth was predicted to be greater in the Hopewell population than in the Indian Knoll population. The data on radius width show this to be true. However, to fully test the hypothesis it must be shown that bone width and diet are related and that significant nutritional differences exist between the two populations. Garn

(2) has shown that bone width is related to caloric sufficiency to the extent that average bone diameter or width may be 15% narrower among the malnourished of a population. The data in Tables 1 and 2 are taken to indicate significant nutritional differences.

The archaeological data also suggest nutritional differences. Excavation of the Indian Knoll site has categorically demonstrated that this population was pre-agricultural (8). Farming implements such as shell hoes and floral remains of domesticated species are absent. The remains of shellfish and mollusca were found interspersed throughout the refuse or cultural debris of the site. The heavily forested area around the Green River provided the Indian Knoll people with a variety of foodstuffs. Remains of hickory nuts, walnuts, and acorns are reported by Webb (8) along with hammerstones which were employed to process the nuts. An abundance of deer, *Odocoileus virginianus*, indicates its importance as a meat source. In fact, of 27,756 animal fragments identified, 23,177 were from *O. virginianus*. Atlatls or spear throwers were found in association with burials and probably were used to kill both deer and wild turkey, *Melaegris gallopavo*.

The Green River provided staples other than just mollusca, for fish were also a significant component of the diet. Webb (8) describes the deer ulnae and tibiae which were fashioned into fish hooks. Skeletal remains of the drumfish, *Aplodinotus grunniens*, were scattered throughout the debris of the midden. Migratory waterfowl such as the Canada goose, *Branta canadensis*, were also found.

The Middle Woodland Period of North American prehistory emerged at least 2,000 years after occupation at Indian Knoll was terminated. The former Period, then, is represented by cultures which benefited from a longer experience adapting to the New World environments. The hunting and gathering society of Indian Knoll did not alter the normal habitats of the natural flora and fauna. As stated above, shellfish were merely gathered, nuts collected, and deer hunted. In contrast, the later Early Woodland cultures which antedate the Middle Woodland cultures actually modified the floral environment to their own advantage. Various excavations of Early Woodland sites have shown remains of plants not naturally indigenous to those areas. Archaeologists infer that the Indians intentionally introduced species whose existence depended upon human management. This took the form of cultivation and selective removal of other plants which might have been competitors. These plants used by man are cultigens, the earliest of which were: *Helianthus annuus*, sunflower; *Iva* sp., marsh elder; *Cucurbita pepo*, squash; *Lagenaria siceraria*, gourd; and *Chenopodium album*, goosefoot or lamb's quarters.

An Early Woodland Kentucky site near Indian Knoll has yielded human fecal material which reflects subsistence activities. Watson (7) reports that the feces at Salts Cave contained hickory, acorn, sunflower seeds, *Chenopodium*, and *Iva*. The latter three probably represent the earliest cultigens in this eastern woodlands area. Significant too were remains of two tropical exotic plants, squash and gourd. Cultivated plants made up 45% of the bulk of the Salts Cave feces. The intermediacy of

this site relative to the Indian Knoll and Hopewell cultures has been shown by radiocarbon dates of 710 ± 200 B.C.

The original stimulus to cultivate was probably a product of cultural diffusion from Mesoamerica where, for example, squash, gourd, and corn are indigenous. The adoption of incipient agriculture in the Early Woodland Period provided the Hopewellians of the Middle Woodland a tested, successful behavioral adaptation. The evidence is impressive that by Middle Woodland times, horticulture had become widely practiced in both the Ohio and Mississippi River Valleys where it was to persist until contact times.

The village site that adjoined the mounds which contained the Hopewell skeletons is today covered by a town and highway and therefore beyond archaeological recovery. Examination of other Middle Woodland sites can, however, provide a picture of Klunk-Hopewell subsistence activity. The McGraw site of Ross County, Ohio, is situated in the flat bottomland or flood plain of the Scioto River. Such riverine locations, even today, are the best areas for agriculture. Prufer (4) reports the discovery of a medium-sized ear of corn in the middle stratum of the site. One may posit, therefore, that, following in the tradition of the Early Woodland, Hopewellians were introducing and propagating alien plants. Prufer maximizes the significance of the corn and believes that this community's primary economic activity was farming based on a shell hoe technology. Secondary activities in order of decreasing importance were shellfish collecting, hunting, and wild plant collecting. Prufer reasons that the nearby burial mounds, which are large in size and rich in material content, could have been built only with the support of a stable agricultural economy.

Middle Woodland sites in Illinois have also yielded corn. Vickery (6) reports finds from the Jasper Newman site of Moultrie County (50 ± 140 B.C.) and the Macoupin site of Jersey County. In Calhoun County where the Klunk-Hopewell site is located, the Peisker site (180 ± 130 , 70 ± 120 , 250 ± 20 A.D.) and Ansell site (250 ± 150 A.D.) also contained corn. Both Illinois and Ohio Hopewell climates were capable of supporting the corn plant.

Judging from the above village or occupation sites, one can categorically state that Hopewellian Indians of the lower Illinois River Valley had expanded the exploitative potential of their environment through farming activities. This is not to say, though, that the Valley itself was not already naturally endowed with a diversity of food resources. Struever (5) has shown the easy availability and abundance of hickory nuts and acorns; *Iva* and *Chenopodium*; deer; geese and ducks; and fish. Both population density and residential stability increased during Hopewellian times. The number and size of burial mounds attest to the population increase while numerous storage pits and earth ovens in close association with a house at the Apple Creek site in Madison County, Illinois, attest to the residential stability. This increase in population density and residential stability is taken to indicate the larger and more varied diet of the Hopewell people over that of their forebearers, the Archaic Indians of Indian Knoll.

The larger and more varied diet of the Hopewell people is considered to be the primary variable which accounts for their bone growth being greater than that of the Indian Knoll people who did not supplement their hunting and gathering with part-time agriculture. There is the possibility, though, that genetics is partly involved. The effective breeding size of the two populations was small. Inbreeding was therefore probably operating in both and contributing to an increase in homozygosity and decrease in overall genetic variability. Since Hopewell populations were larger and since they participated in an extensive trade network, outbreeding was probably more characteristic than in earlier Archaic times. If outbreeding were more common among Hopewellians, their larger bone diameter could be partly explained. For example, hybrid vigor in the form of increased stature has been observed among the offspring of outcrosses between European sailors and Polynesian women. This is not taken, however, to detract from the significant nutritional differences between the two populations.

Conclusions

1) The diet of a Middle Woodland-Hopewell population from the lower Illinois River Valley has been shown to differ from that of an Archaic population from the Green River in Kentucky. The latter population relied exclusively on hunting and gathering while the former enjoyed a larger and more varied diet due to the adoption of part-time agriculture to supplement hunting and gathering.

2) Statistically significant differences in radius diameter or width were shown to exist both inter- and intrapopulationally. Males of both populations were larger than females. Males and females of the Hopewell group were larger, respectively, than males and females of the Indian Knoll-Archaic group. These population differences in bone diameter are attributed to differences in the aboriginal diets.

Acknowledgements

I would like to thank the late Dr. G. K. Neumann of Indiana University, who provided some of the skeletal materials used in this study, and Dr. C. Conrad Johnston of the Indiana University Medical Center, who provided me access to the photon absorptiometric apparatus. Also, I am especially in debt to Dr. David M. Smith of the Medical Center and Dr. Robert J. Meier of Indiana University who helped me with technical aspects of this study.

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The McKinley Site

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Abstract

The McKinley Site is located just south of Noblesville, Hamilton County, Indiana. It was first discovered in 1937 when a road was widened. It was excavated by Downey Raibourn and Jack Householder, as a salvage operation, starting in 1953 under the direction of the late Glenn Black.

The site had three periods of occupation from Late Archaic to Early Woodland and finally during the contact period of 200 years ago. In the Late Archaic there was evidence for hot-rock cooking plus an emphasis on small animal hunting and gathering. The tool inventory was more sophisticated than for later occupations. The Early Woodland period emphasized larger animal hunting and there was evidence for pole structures. The surface and near-surface occupation included stone artifacts and tools as well as historical objects. Eighteen burials recovered showed associations with Indian Knoll.

Introduction

The McKinley Site, located just south of Noblesville in southeast Hamilton County, was a salvage operation, excavated as a result of Indiana State Highway operations.

In 1937 the road leading to Noblesville from the south was a very narrow, paved highway. When the highway was widened, the road scrapers cut into the east bank of the river terrace just south of a house owned by Mr. and Mrs. McKinley. Bone fragments and flint chips were noticed by the highway crew, and Dr. Glenn A. Black, Indiana University, was notified. He and Jack Householder examined the site and noted the situation. Householder visited the site a number of times as the years passed and collected samples from the surface.

Downey Raibourn and Jack Householder began excavation of the McKinley Site on June 7, 1953. Of the total area dug, approximately half of the work (750 square feet) was finished in the first season to sterile soil, which in some cases went over 3 feet deep. In the next 2 seasons approximately 775 square feet of surface was excavated to sterile soil.

The author wrote up the McKinley Site excavation as a thesis at the suggestion of Dr. James Kellar, as part of the program to finish the work of the late Dr. Glenn Black.

Site Description

The McKinley Site overlooks to the west, what is known as Horse Shoe Prairie which is a fertile, flat land formed by several sharp bends in the west fork of White River. In all probability during Late Archaic-Early Woodland times, the river bank came up to the terrace that is contiguous to the site. It has apparently been an ideal location for occupation since post-glacial times.

The area of the site had, in the immediate past, been in garden, but at the time excavation began, it was covered with broom grass, wild lettuce and weeds. The plow zone penetrated to at least the 0.4 foot level, but there was still much material on and near the surface. In this level were found bones of domesticated animals such as sheep and hog, glass and other artifacts of both modern man and of early settlers.

The area of occupation extended for 60 feet along a northeast-southwest line and roughly paralleled what had been the original bank of the river as it made its sharp bend forming the prairie. The road cut doubtless destroyed much of the site, but there is no estimate of how much. Unsuccessful attempts were made to locate material west of the right-of-way. The 60 lineal feet previously mentioned applied to the surface and to the layer down to the 1.6 foot level. The third occupational area was primarily limited to a more circular area of about 30 feet in diameter at the north end of the 60-foot line.

Methods

The site was laid out in 40 squares, each 10 feet by 10 feet. During the first season, excavation was continued to sterile soil on a 10 foot block plan as originally intended. In addition, two 5-foot blocks were opened 50 feet north and 30 feet east of the center lines to establish the perimeter of the site. However, at the start of the second season the expedition was constantly hampered by after-hour pot hunters. For this reason, 5-foot squares were excavated and every effort was made to complete a square before opening another. This was not an ideal technique, and has led to many problems in studying the field notes, but was necessary procedure under the circumstances.

In the laboratory, the author analyzed the projectile points for their discrete attributes under a formula worked out at the University of Michigan (1), as follows: outline of the blade, outline of the base, junctures of the haft element, presence or absence of a shoulder or a barb, outline of the shoulder element, tang form, tang outline-proximal segment.

Of the 157 total selected points, 69 were complete enough for metric measurements.

Results

The stratigraphy of the site was not apparent at the time of the excavation, but analysis clearly indicated three occupations. On the surface, along with the modern debris, a few sherds of grit tempered cord marked pottery of Late Woodland variety were found. However, many of the projectile points in the same context were Archiac. Under this modern debris area which can be classified with the surface as Zone C,

a continuous black layer was noted over the entire site and was variously referred to in the field notes as black soil, black debris, black disturbed and mottled. It varied in thickness from several inches to 2 feet, and averaged about 1 foot. This fact seems to correlate well with the second occupation, or what was called Zone B. The extreme blackness of the second layer indicated an intense occupation.

Under Zone B in discontinuous patches of about the same thickness was a lighter-colored occupation that was called yellow-disturbed by the excavators. This first occupation of the site was called Zone A. It is not as wide-spread as Zone B and was similar except for the presence of many shell fragments and a change in the style of the projectile points. Other artifacts of stone and bone also indicate a change of style. Below Zone A was a sterile yellow Miami silt loam soil.

The chipped stone projectile points recovered at the McKinley Site were well distributed over the area. In all, some 363 points and fragments of points were recovered, but only 149 were complete enough for the discrete attribute analysis. The raw material was for the most part chert. Eight bone points were added to this analysis for a total under consideration of 157. Of this total, 64% were found on the surface, 24% in Zone B and 16% in Zone A, including the bone points.

TABLE 1, *Stone and bone implement inventory.*

	Zones		
	A	B	C
Stone Tools			
Anvils	0	3	16
Hammers	25	24	3
Axes:			
$\frac{3}{4}$ grooved	0	2	0
full grooved	0	0	4
Net Sinkers	0	4	0
Pestles:			
elongated	2	0	0
bell shaped	4	0	0
Knives	1	5	10
Scrapers (4 types)	3	6	23
Drills	0	13	20
Smoothing stone	0	1	0
Bone Tools			
Awls	29	43	—
Bodkins	9	0	—
Needles	2	11	—
Atlatl hook	1	0	—
Fish hook	1		
Ornaments and Problem Forms			
Banner stone fragments	0	0	3
Stone tube	0	0	1
Stone beads	1	0	0
Shell beads	3	0	0
Perforated turtle shell rattle	1	0	0
Bone tubes	0	2	0

My analysis revealed at least one new type of projectile point for each Zone. In Zone A, two distinct types were noted. All bone projectile points were found in this lowest zone, and were similar to those found at Indian Knoll (4). In the classification, they were described as having an ovate blade and a lateral-lateral haft element (also known as side-notched). A type originating (3) in Zone B was described as having an excurvate blade and straight shoulders. Only Zone C revealed triangle points with subconcave bases.

The tool inventory was very extensive and was divided into two general categories, stone and bone (Table 1). Of special interest was the fragment of an atlatl hook, which was found at the lowest level. Atlatl hooks were known to have been used in the area during Late Archaic times (2). The bone material recovered was, for the most part, in good condition and quite extensive (Table 2). The bones and bone fragments from animals used for food. Included were disarticulated dog bone fragments found scattered throughout the site.

TABLE 2. *Subsistence patterns.*

Bone	Number of pieces	
	Zone A	Zone B
Deer— <i>Odocoileus virginianus</i> ¹	765	1,625
Turtle— <i>Amyda spinifers</i> ,	226	72
<i>Chelydra serpentine</i> and		
<i>Terrepene carolinensis</i>		
Ground Hog— <i>Marmota monax</i>	43	11
Squirrel:		
Fox— <i>Sciurus niger</i>	—	9
Gray— <i>Sciurus carolinensis</i>	—	41
Raccoon— <i>Procyon lotor</i>	11	22
Beaver— <i>Castor canadensis</i>	8	—
Rabbit— <i>Sylvilagus floridanus</i>	5	5
Opossum— <i>Didelphis virginianus</i>	—	1
Muskrat— <i>Ondatra zibethica</i>	47	3
Rodent misc.	—	10
Shell	60+	—
Bird:		
Small	38	—
Large	8	—
Dog— <i>Canis familiaris</i>	3	3
Unidentified (28 burned fragments)		

¹ Representing an animal count of 84, using the Dick Adams method, Indiana University.

Of the 18 burials recovered, 8 were probably related to the Zone B occupation and the remaining 10 were from the deepest level of occupation. All heads were oriented facing south except one which faced north and

showed evidence for red ocher in the pelvic region. There was one male-female double burial.

Most of the burials were on their back with flexed lower limbs. Exceptions included two fully extended burials, and one unusual burial which was on its back, fully extended to the knees, then with the lower legs flexed back under the body. Probably the most important burial was a male, found at the lowest level, tightly flexed and buried face down. Associated with it was a perforated carapace rattle very similar to those found at Indian Knoll in Kentucky (4).

TABLE 3. *Age comparisons between McKinley and Indian Knoll.*

Classification	Percentage	
	Indian Knoll	McKinley
Newborn to one year	6.11	11.00
1 to 3 years	19.80	6.00
Children 4-12 years	14.13	0
Youths 13-17	6.64	6.00
Near Adults 18-20 years	.28	0
Young Adults 21-35 years	31.64	22.00
Middle Age 36-55 years	4.32	39.00
Old Adults 55 + years	.02	11.00
Unidentifiable	9.10	0

Of the 18 burials, there were 10 males, 5 females, and 3 infants. The age at time of death ranged from 13 to 51 for the males and from 30 to 68 for the females. The final skull measurements were made by the late Dr. Georg Neumann, and are very similar to Indian Knoll. However, the McKinley population lived longer on an average than the Indian Knoll population and were 1 to 4 inches taller. (Tables 3 and 4).

TABLE 4. *Comparison of stature between Indian Knoll and McKinley.*

Zone	Burial No.	Age	Sex	Manouvrier	Pearson	Neumann
A	12H1-13	13	M	1580	1569	1622
A	12H1-11	29	M	1689	1667	1682
A	12H1- 7	30	F	1575	1557	1591
A	12H1-12	40	M	1738	1730	1722
A	12H1- 9	45	F	1673	—	1640
A	12H1-18	46	M	1630	1618	1652
A	12H1-10	46	F	1568	—	—
A	12H1- 6	67	F	1575	1557	—
B	12H1- 3	32	F	1548	1527	1562
B	12H1- 5	44	M	1693	1693	1698
McKinley Average			F	1588	1542	1588
McKinley Average			M	1688	1677	1688
Indian Knoll Average			F	1569	1532	—
Indian Knoll Average			M	1656	1640	—

Discussion

Other interesting features, in addition to burials, included pits and post holes. There appeared to be no consistency in the size and shape of the pits. There was no evidence for bark or rock lining for storage purposes although several small pits were found containing charred nuts. Other small pits contained charcoal and red burned clay.

One special pattern found in the lowest level of occupation was noted by the excavators. At least six pits were uncovered with concentrations of rocks in the center. These pits ranged from 1 to 2 feet in diameter, and averaged a foot deep. In many instances these pits showed a possible flanged rim. The excavators indicated that this was evidence for "hot rock" cooking by the earliest occupants of the site.

Because of the density of the occupation it was difficult to identify all of the post holes that were present. Some organization was present, *e.g.*, a suggestion of a straight line in the southwest quadrant of the excavation. The most intensive occupation appeared to be in the lowest strata of Zone B. The organization that was present in the post holes can probably be associated with the housing used primarily by the Zone B occupation.

Conclusions

It is obvious that hunting and gathering was the life style throughout the occupations of the site, and that the availability of the fauna was reasonably constant. However, there seemed to be an emphasis on small animals at the lowest level, and a corresponding emphasis on larger animal hunting in the middle occupational level.

There seems little doubt that the earliest village in Hamilton County, Indiana, may well have been a small band of people, who left their home at Indian Knoll, Kentucky, and worked their way up the White River to settle at Horse Shoe Prairie sometime before 1000 B.C.

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The Hybrid Origin of the Arikara Indians

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Abstract

The hypothesis of a hybrid origin of the historic Arikara of the Great Plains was examined by multivariate-discriminant computer analysis applied to samples of Arikara and related skeletal material. The ethnohistorical and archeological evidence bearing on the Arikara was summarized, and the skeletal material utilized was described. Three putative ancestral groupings were examined by F and t significance tests for cranial measurements and indices, and were subjected to multivariate analysis with Arikara sample series to determine their relationships. The initial hypothesis of the Arikara being closely related to the protohistoric and historic Pawnee, and less closely related to Siouan groups such as the Mandan and the Ponca and Omaha, was supported.

The purpose of this study was to examine the hypothesis of a di- or tri-hybrid origin of some of the populations of the Great Plains, as initially advanced by the late G. K. Neumann in 1942 (5). This hypothesis was tested through the application of multivariate computer analysis. The Caddoan-speaking Arikara of South Dakota were utilized as an example. In their migration up the Missouri River, the Arikara came into contact with the Siouan-speaking peoples of the Middle Missouri Tradition. Despite the occasional war-like nature of the contacts, considerable mixture did occur, especially in the northernmost Arikara villages. Contacts of lesser importance may have been made with the Siouan bison hunters of the Dakota and Dhegiha divisions. Physically, the analysis primarily involved populations of the Muskogid and Dakotid varieties, following the terminology of Neumann (6). These groups include: 1) historic and protohistoric Pawnee represented by samples of the Upper Republican and Nebraska Culture aspects and historic Pawnee (Muskogid Ancestral Group); 2) a Plains Woodland and Mandan-like Siouan population (Dakotid A Ancestral Group); and 3) the possibility of significant contribution of a second type of the latter (Dakotid B Ancestral Group), represented by the Ponca and Omaha. It is to be understood that the term "putative ancestral group", particularly in the case of the Dakotid B Ancestral Group, does not infer direct descent, but rather the possibility of mixture through contact. It has long been suggested that the Arikara are descendants of the Pawnee (3, 9, 10, 11, 14). Most of the evidence for this is linguistic in nature. The history of the Arikara prior to the close of the 18th century is not perfectly known, and can only briefly be summarized here. Northernmost of the Caddoan-speaking people, the Arikara were the last of that linguistic stock to come in direct contact with white chroniclers. Archeologically, some time after circa A.D. 100, but still several centuries before the arrival of the first white men in the Central Plains region, the Middle Woodland-Hopewellian complexes were succeeded by a group of semi-horticultural pottery-making cultures implying a much more stable pattern of settlement. Included in these semi-sedentary complexes were several prehistoric manifestations featuring small loosely arranged unfortified settlements—

the Nebraska Aspect of northeastern Kansas and eastern Nebraska, and the Upper Republican Aspect of western Kansas and Nebraska (16). There is archeological evidence that the Arikara were established in present central South Dakota by the 15th century, as represented by the Initial Coalescent Variant (4). The Initial Coalescent sites appear to represent the first stage of an amalgamation of the Central Plains and Middle Missouri into the Coalescent Tradition. There is a considerable gap in the archeological record between the Upper Republican and Nebraska Cultures and the Pawnee, perhaps due to the dry years of the 1400's in the Central Plains. The close similarity in houses, pottery, and other artifacts between the Initial Coalescent and the Central Plains Tradition however leaves little doubt that the Initial Coalescent groups were immigrants from the Central Plains. Few studies in physical anthropology have been done on the remains from the Great Plains. Wedel (15) says of this area: "For various reasons it has remained one of the last major geographical provinces to attract the interest of trained students of prehistory—strange to say, despite the demonstrable richness of this upper Missouri region for the study of human prehistory, no comparable body of data correlating cultural and somatological materials on the Arikara—or for that matter on any of their neighbors—has yet been published".

Crania of 169 nondeformed adult males comprise the 9 series used for the study. All of the crania were measured and examined by Georg K. Neumann, and the data placed in the files of the Laboratory of Bio-anthropology, Indiana University, prior to his death. The original data is now in the possession of his surviving family. The samples used are well-documented temporally, and are based on specimens whose cultural affiliations are known either from archeological materials or historic records. Identical measurements and observations were employed in assessing each series; error on the part of non-comparable data from different observers was eliminated. The samples represent the totality of available materials which adhere to the above criteria, and are considered adequate for estimations of the parameters of the actual populations involved. The question of the particular characteristics which are similar and different between the groups should hopefully be examined in light of what is known of the heritability of anthropometric traits. However, estimates which have been made show a considerable variability in results, depending on the methods used. Twin studies appear to show a fairly high agreement (12) in that certain traits such as stature, limb lengths, facial height, and the cephalic index appear to be highly heritable, having little within-pair variability among dizygous twins (1, 2, 7, 8, 13). Despite the claim that such studies show agreement among themselves (12), discrepancies do exist. Osborne and DeGeorge (7) find the cephalic index to have the highest heritability, while the same trait is listed as "unstable", inferring a low heritability, in Ostertag's study (8). In a study mentioned above (13), twins were compared in terms of 64 anthropometric traits, of which only 3 were not significant at or beyond the 0.05 level of probability. Of the remaining 61 variables, all but 6 were significant at or beyond the 0.01 level. This implies that nearly all anthropometric traits have very significant heritability factors, while being little influenced by environment. However, the length of the left ear is significant at the 0.05 level, while the right ear

shows considerably more significance ($p=0.01$) between mono- and dizygous twins! Given such difficulties and inexplicable results, it would seem that heritability studies point to very different conclusions. Given also the questionable nature of mandibular traits due to developmental influences, these problems make it unwise, in the opinion of the author, to base any interpretation of the traits which emerge from the analysis on these grounds.

The ancestral groupings were initially arrived at by an examination of the temporal, spatial, and apparent biological similarities of the series involved. Three statistical methods were used to substantiate this consolidation of the material, including analysis of similarities of qualitative variables, F tests of homogeneity of variance, and t tests for significant differences in means. Eighteen multivariate-discriminant functions were calculated, using all possible combinations of the ancestral groups with the two initial Arikara series and the pooled "tribal" Arikara series, for both measurements and indices. The initial separation of the total Arikara sample into two series, the Muskogid Arikara, and the Dakotid A Arikara (after the terminology of G. K. Neumann) was made for two reasons. The available information indicated that the skulls grouped as the Muskogid Arikara originated in earlier, more southerly sites where the population would be more likely to resemble the proposed original parental (Muskogid Ancestral Group) population, and that the Dakotid A Arikara skulls originated in sites later in date and more northerly in the territory occupied by the Arikara, and would be more likely to resemble the groups contacted later in Arikara history (the Dakotid A Ancestral Group). Also an examination of the material by the author and G. K. Neumann indicated that the samples were slightly different in morphological characteristics. The results of the initial multivariate functions appeared to bear out this contention. However, the differences between the Arikara samples was slight, and in the opinion of the author the total Arikara sample, or pooled "tribal" Arikara series was the best indicator of overall relationships. The final multivariate-discriminant functions which deal with this grouping are therefore considered to be the most applicable to the examination of the hypothesis, and are summarized here.

The analysis indicated that the Arikara were most closely related to the Muskogid Ancestral Group, and that this similarity was greatest in the region of the cranial vault, both by size and by proportion. The overall conformation of the facial area was also very similar, including the facial length, and nasal and orbital breadths. The Dakotid A Ancestral Group was the next most closely related grouping, with the similarity greatest in the nasal region as expressed by the nasal height, nasal root height index, and the nasal index. There was some similarity in the facial breadth and the flatness of the face. The Dakotid B Ancestral Group was the least similar to the Arikara. No particular region of the cranium was related as a unit to the Arikara. The greatest similarity between the Arikara and the Dakotid B Ancestral Group was in the high nasal bones, and in a moderate amount of the flattening of the cranial base; the cranial vaults were quite different. This general picture was in agreement with that proposed by the original hypothesis. The relationships between the

ancestral groupings and the Arikara may be summarized by the use of a generalized multivariate D^2 value, considering all variables, which indicates general biological distance between populations. To place these values in perspective, if the statistic were applied between the Arikara and a remotely related American prehistoric population such as the Indian Knoll Archaic series from Kentucky, a value of $D^2 = 24.00$ would obtain. The close relationship between the Arikara and the Muskogid Ancestral Group was reflected in a D^2 value of 4.65. The Dakotid A Ancestral Group was not far behind, with a D^2 value of 4.90, and appeared to have contributed significantly to the historic Arikara. The Dakotid B Ancestral Group was the least related of all the putative ancestral populations tested, but the dissimilarity was not great; $D^2 = 5.13$. In this regard, the Dakotid B Ancestral Group appeared to be close enough to retain it as a viable putative ancestor, a question which was left open in the initial formulation of the hypothesis.

The available evidence, as summarized here, appears to lend considerable credibility to the hypothesis of a hybrid origin of the Arikara. Certain sets of characters which serve to identify the samples employed have been identified, and may serve as a guide to workers planning further craniometric studies on similar material.

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Excavations at the Daugherty-Monroe Site, 1971

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Abstract

The 1971 Indiana State University Field School excavated, for the second season, the Daugherty-Monroe site (12-Su-13) in northern Sullivan County, Indiana. The site contains both Allison and LaMotte cultures. Results of tests made in two mounds were largely negative. Excavations in the more southern village area turned out to be pure Allison. Distinctive traits typical of the Allison culture included house patterns, storage/refuse pits and projectile points—a variant of the Lowe Flared Base type. Bone artifacts and refuse were rare compared to LaMotte deposits to the north, but this can probably be attributed to an acid soil in the area excavated.

Introduction

The purpose of the excavations at the Daugherty-Monroe site was to gather additional information on the LaMotte and Allison cultures (1). Excavations were carried out by the Archeological Field School directed by the late Dr. Edward V. McMichael.

The site is located some 30 miles south of Terre Haute, Indiana, on the second terrace of the Wabash River in Sullivan County, east of Hutsonville, Illinois. Before the 1970 summer excavations, it was believed that the site had been occupied only by people of the LaMotte culture (2). It was not until the last few days of the field school that an Allison component was discovered (1). Initial attention was diverted to a group of mounds in a wooded area near the LaMotte-Allison village. Approximately 13 mounds exist in the wooded area 50 yards to the northeast of the main site. Mounds C and H were selected to be tested.

A trench was opened across the center of Mound C (Fig. 1). It was found that a primary clay mound, approximately 15 feet diameter and 26 inches deep, had been built, then a later secondary sand mound added. The clay, according to personal communications with Mr. Cantin of the Indiana State University Geography and Geology Department, was from a fluvial deposit. A skelton of a horse, buried about 50 years ago, was found in the center of Mound C. Three Allison potsherds, numerous fire-cracked rocks and flint chips were found in the burial fill. No other features were found in Mound C.

Mound H rose some 27 inches over its base and proved to be a single structure, with loading of sandy soil. Artifacts recovered consisted of a number of Allison Cordmarked sherds and one Baumer Fabric-marked rim. These were apparently carried in with the fill. There were no post molds or other features to indicate the function of the structure. While

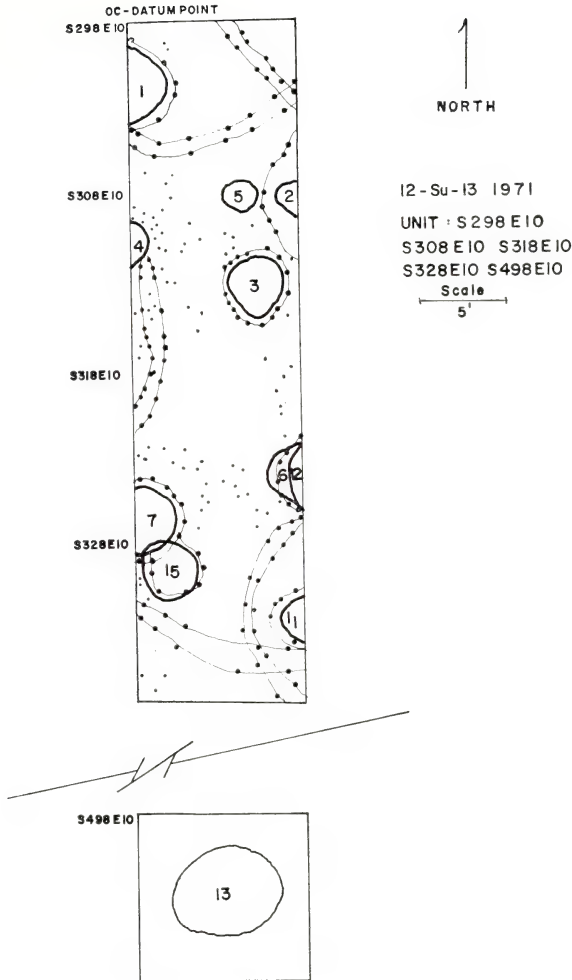


FIGURE 1. *Horizontal plot of features at 12-Su-13 (1971).*

the excavation did demonstrate that Mounds C and H were man-made, no conclusion can be drawn as to their use.

The Village

Excavation of the southern edge of the village demonstrated that only Allison occupation was there. Four 10 x 10 foot units were opened, some 300 feet south of the 1970 excavations. Altogether, 10 basin-shaped pits, averaging about 30 inches deep and 36 inches in diameter were found. All the pits, except Features 5 and 13, had a single row of post molds around them. The postmolds averaged about 6 inches apart and 3 inches in

diameter. These posts may have supported a superstructure used as protection. All of these were storage and/or refuse pits.

One large refuse pit deserves special attention. This pit, Feature 13, was found in a 10 x 10 foot test unit, 200 feet south of the other 1971 excavations, while searching for the village perimeter. The pit contained numerous Allison sherds. Apparently this was a refuse dump located on the edge of the village away from the main house areas.

Portions of possibly six house patterns were also found in the village. Each pattern consisted of a double row of post molds approximately 12 inches apart and with posts measuring 3 to 4 inches in diameter. At the present time it is unknown whether or not the pits were used in or outside these house structures (Fig. 1).

Artifacts Analysis

Artifacts from Mound C consists of three Allison Cordmarked sherds.

The artifacts from Mound H consist of the following sherds: 195 Allison Cordmarked; 6 Embarras Simple Stamped; 1 Baumer type sherd with interior and exterior fabric marks and a squared rim; and 1 piece of deer leg bone. This material all seems to be Allison (except for the Baumer rim sherd) (Table 1).

TABLE 1. *Archeological materials recovered, 1971.*

CERAMIC	
2,190 Cordmarked (53.2%)	44 Simple Stamped (1.1%)
1,038 Smoothed Over Cord (25.2%)	716 Unclassifiable (17.4%)
93 Cross Cord (2.3%)	33 Other (0.8%)
CHIPPED STONE	
6 Lowe Flared base projectiles	1 Trianguloid knife
4 Scrapers	1 Guilford-like projectile
4 Lamellar flake knives (Harrison County flint)	1 Trianguloid projectile
2 knife fragments, 1 parallel sided	
STONE	
1 Nutting stone	1 Celt
1 Abrader	2 Crinoid stems used as beads
OTHER	
1 Fired Clay object (resembling a claw or beak)	1 Copper pin (2.5mm)

The ceramics found in the village during the 1971 excavations are predominantly Stoner Cordmarked along with a few simple stamped sherds of the LaMotte "Embarras" type (3). There were a few aberrant sherds

present, including: 3 cordmarked zoned, 5 simple and check stamped zoned, 1 sherd with interior and exterior cordmarking with the interior marking perpendicular to the exterior, and 6 temperless "toy Pot" sherds.

A study of the rim sherds has shown that 80.7% of the cordmarking is vertical to the rim (Table 2). The cordage ranges from loosely to tightly twisted, and from close spaced to slightly over 5 mm apart. Coil fractures were found on many sherds. Firing was uneven, resulting in both oxidation and reduction on the same vessels. The tempering of the sherds is fine to medium sand and grit.

TABLE 2. *Rim treatment compared to surface treatment.*

	Cordmarked	Zoned	Unclassifiable
Reed punctate	21	—	—
Smoothed	23	—	2
Oblique notching	7	—	—
Scalloped	5 ²	—	—
Notched	8	—	—
Scalloped & notched	3	—	—
Interior notching	1	5	2
Other ¹	4	—	—

¹ Includes one tapered, three interior and exterior notched.

² Probably part of vessel both notched and scalloped from Feature No. 13.

Conclusions

Although the authors regard the LaMotte culture as a direct descendent of the Allison culture, some variations were noted during the 1971 field work.

No house post mold patterns have been observed in the LaMotte section of the village, even though a great number of random post molds were found. The only house pattern found associated with Allison was a "D-shaped" pit house. From work on the Illinois side of the Wabash, Denzil Stephens suspects large circular house patterns for LaMotte. There were no post molds associated with the LaMotte pits, unlike what proved to be characteristic of Allison.

LaMotte pits were generally much deeper, more straight-walled and oval to circular, although their diameters were about the same as Allison. Allison pits were semi-conical, while the LaMotte pits were usually basin-shaped. The deeper LaMotte pits may suggest an increase in population and consequently an increase in storage problems.

A diagnostic trait of the Allison culture is Stoner Cordmarked pottery and for LaMotte, Embarras Simple Stamped pottery. A large quantity of bone tools were found this summer in the Allison excavation. A difference has also been noted between the Allison and LaMotte point types. Both used Lowe flared base point made of Harrison County flint, but LaMotte points appear to have been reworked.

Recent results of Carbon-14 dating has yielded dates of A.D. 570 and A.D. 540.

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A Provisional Taxonomy of Prufer's Scioto Tradition

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Abstract

Indiana and Ohio archaeologists have been reluctant to classify and culturally affiliate archaeological remains. A symptom of this unwillingness to classify is the forced definition of large, vague and invalidly-defined units such as *Hopewell*, *Adena* and *Fort Ancient*. The purpose of this paper is to abolish these terms as culture taxons by proposing a classification of Scioto Tradition phases.

For various reasons of historic, scholastic, and idiosyncratic nature, Indiana and Ohio archaeologists have been loathe to define cultural affiliations from archaeological remains, that is, do archaeological taxonomy. It should be noted that McKern's (9) Midwestern Taxonomic System was developed on the basis of materials in the northern Mississippi Valley area, and was only nominally applied in Indiana and Ohio. Western Indiana is outside the range of problems discussed in this article, but terms used there are mostly borrowed from Illinois archaeology. Prufer's recent work in Ohio, however, has radically altered established views of conventional classification schemes there.

A symptom of this unwillingness to label is the forced definition of large, vague, and invalidly defined units. Such terms as *Hopewell*, *Adena*, and *Fort Ancient* hang on to plague contemporary comparative archaeological study. The purpose of this article is to rectify past sins, and, at least, to abolish two of these terms as taxa (*Hopewell* might be maintained in a ceremonial complex status, with its "Interaction Sphere," and/or a pottery type designator—but not as a cultural unit). For an up-to-date treatment of *Fort Ancient* see Prufer and Shane (15). Part of the reluctance to classify Midwestern Woodland materials is that so little data are available from living areas. Ceremonial remains do not seem to be sensitive spatial indicators, probably due to the rapid diffusion of associated religious concepts, as materials related to subsistence technologies.

Using the classification precepts of Willey (19) Prufer has proposed a "Scioto Tradition" (12). This concept is based on the belief that there is a continuity of development in the Ohio Valley of basic Woodland character which is largely unknown due to absence of detectable living sites. This assumption is not accepted by all workers in the field. The term *Scioto* is unfortunate in that it includes southeastern Indiana, southern and central Ohio, north central and eastern Kentucky, and western West Virginia besides the focal Scioto River drainage of south central Ohio (16, p. 212 and Fig. 3, p. 218; 17, p. 137). A specific site name or, perhaps, an appropriate descriptive geographical term should have been chosen. The term *Scioto Hopewell*, rather than *Ohio Hopewell*, does have some geographic plausibility though, in that the Scioto basin is the climax area, and manifestations of this development, though largely restricted to Ohio, do occur in some adjoining states.

It is very dangerous to place time limits on archaeological units, but the beginnings of the Scioto Tradition appear around 2000 B.C. The tradition is completely prehistoric, being displaced by Late Woodland and Mississippian populations, such as those of the Fort Ancient Tradition.

Along with Scioto, there are a number of Woodland traditions in the eastern United States which in some stage of development manifest a strong cast of Hopewell ceremonialism (5, Fig. 3, p. 181). It is believed that Hopewell ceremonialism evolved within the Havana Tradition of Illinois.

Although Prufer has described "Scioto Hopewell" in various publications, a set of traits defining the Scioto Tradition as a whole has yet to be put forth. In fact the literature suggests that Scioto Hopewell is confused with Scioto Tradition. Struever's map (16), for example, should read Scioto Hopewell for Scioto Tradition, and his definition of Scioto Tradition refers to Scioto Hopewell. It must be realized that Prufer's approach to this material has been from his initial interest in "Classic Hopewell" (11). The integrating feature of the tradition as perceived by Prufer appears to be cordmarked utilitarian pottery. Scioto can be contrasted to the Havana Tradition by the presence of extensive ceremonial cremation and a deciduous forest setting.

Table 1 represents a taxonomy of the Scioto Tradition, with units comparable to what Prufer calls phases, following procedures characteristically applied to the Southwest United States. These are essentially "generations of pottery makers" (6, p. 98), in river valleys. Local variants of what Prufer (13, p. 49), calls Early Middle, Late, and Latest [Scioto] "Hopewell" are, therefore, assumed to be components, though generic terms are not given. The table is not complete as regional phase sequences can probably also be established for the Miami and Muskingum drainages in Ohio. Selective explanation of phase terms proposed seems required. The most striking feature is the complete absence of the term *Adena*. Early Adena is replaced by *Fayette*, from Fayette Thick pottery (4). *Robbins*, from Dragoo's Robbins Complex (3, p. 269), has become the term for Middle and Late Adena. Fayette is too generalized to divide into phase units at this time. It differs from Munson's (7) Marion Culture of the Havana Tradition in that pottery is typically decorated by cordmarking, rather than by fabric impressing. Fayette and Marion pottery is quite thick. Michael J. Rodeffer (personal communication, 1972) is of the opinion that, on the basis of burial evidence, the dichotomy between Fayette and Robbins is not apparent in the Licking basin, and proposes a general Early Scioto Hartman Phase. The writer believes, though, that ceramic evidence will eventually indicate a division of this unit. *Westenhaver*, following Black's thinking (2, p. 301), is called Adena by Prufer (14, p. 130), and *Charleston* is called Adena by McMichael (10, p. 37-8). This writer is terming Prufer's Hopewell Phase McGraw (after Prufer's McGraw site) (14). New Castle, rather than Mounds or Anderson, is used for Indiana Middle Scioto because of the recorded presence of Hopewell pottery (18). Prufer's term *Peters* (14) seems preferable to Baby's *Cole* (1), since it is defined on the basis of pottery. An intrusive infant burial (8) was extracted from Earthwork Four, West Mound, at the New Castle site, indicating possible

TABLE 1. *Selected regional phase sequences of the Scioto Tradition.*

	INDIANA Whitewater drainage ¹	OHIO Scioto drainage	WEST VIRGINIA Kanawha drainage	KENTUCKY Licking drainage
Late Scioto	"Little Blue"	Peters	Buck Garden	?
Middle Scioto	New Castle	McGraw	Armstrong Murad	Wright
Early Scioto	Robbins Stage Nowlin	Westenhaver	Charleston	
	Fayette Stage			Hartman

¹And environs.

evidence of Late Scioto occupation in east central Indiana. The term "Little Blue," from the nearby Little Blue River, is tentatively coined here to represent this manifestation and the phase it represents. Excluding the term *Charleston*, McMichael (10) is followed for Kanawha valley phases. McMichael's phrase Kanawha "Tradition" should be replaced with Kanawha Regional Sequence.

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Multivariate Analysis and Human Skeletal Populations

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Abstract

The problems of determining the relationships of archeologically-derived human skeletal populations are discussed. Multivariate analysis is examined in terms of its statistical basis, its value as a tool in studies of human skeletal populations, and its development and history of application in anthropology. The specific technique of multiple-discriminant analysis and its application to problems of microevolutionary change is discussed.

One problem in studies of populations based on archeological sites is delimiting the population's boundaries. This may be done on the basis of grouping skeletal materials according to association with particular assemblages of artifactual materials. If different populations resemble one another in a number of diagnostic characteristics, they may be combined into larger groupings. Temporal boundaries are established with the aid of analysis of differences found in cultural materials at different stratigraphic levels, and from the dating of specific periods in the overall complex. Both temporal and spatial delimitation provide the framework for the establishment of relationships of the population. The remains of a specific culture are assumed to constitute an intrabreeding population which may be characterized by a cluster of characteristics and form a recognizable stabilized entity, whose pattern of variation is known. Only after analyzing the physical characteristics of the available remains and comparing the results with other delimitable populations can particular samples be identified as having a certain likelihood of relationship with a particular archeological population or well-defined tribal grouping. Since one purpose of many studies is the selection, identification, and evaluation of traits having diagnostic phyletic significance for the samples involved, attention must be given to the selection of traits that have historic meaning at a particular level of differentiation. The premise is that these traits, and functional combinations of them, will best reflect the genetic continuity of the populations in accord with other evidence. Once traits have been identified, the populations may be compared to a number of other series. It should be emphasized that combinations of traits rather than individual traits, whether continuous or discontinuous in nature, serve to delimit and identify a group.

Of the characteristics which can be studied in prehistoric bone material, little is known of the specific genetic factors involved. Despite this limitation, in almost every case nothing else remains of the biology of early man, and as much reliable and useful information as possible must be obtained. It is apparent from the nature of the patterns of distribution of the values of the measurements and observations that these traits are polygenic in nature. Although the genetic and environmental components

are not presently quantifiable, this variation is in no way detrimental to the use of these traits for comparative purposes; it is rather the nature of varietal groupings to exhibit a degree of overlapping in their characteristics, and the statistical methods employed in descriptive and inductive presentations in general make use of, assess, and in fact require this variation for their proper application. Indicial traits, demonstrating an absolute relationship of proportion between two measurements, may be employed along with measurements and observations. Change on a varietal level may occur in the form of an overall reduction or increase in gross size, with the form of the cranial proportions remaining constant. This has been demonstrated for Amerind varietal groups (1), and may reveal relationships that are not obvious from measurements alone.

In an analysis that deals with relationships and similarities of human skeletal populations it is appropriate to use a tool such as multivariate analysis, which considers the total physical variation present, and assesses the relative contributions of each variable in distinguishing between groups. A condition which must be met for such an analysis is that the same kinds of data be collected for all individuals of all groups under consideration. Assumptions which must be made are that the various samples have a multivariate-normal distribution, a common dispersion matrix, and a linear correlation between variables. A multivariate analysis is characterized by its consideration of a number of characteristics simultaneously in assessing the relationships or discriminating traits between two-or-more groups or sample series. The important distinction of this statistical approach is that the totality of biological variables are considered in combination, as systems. The interrelationship of all the morphological characteristics of the individual and the relative significance of each variable is considered. The significance for evolutionary change of individual traits can only be assessed in terms of their relationships to other traits. All biological characteristics interact. A bone or a tooth or a skull is a unit, and should be dealt with as such rather than as an assembly of individual traits. Bronowski and Long (4) said that the correct statistical method for the study of discrimination in anthropology must treat the set of variables as a single coherent matrix, a capsule summary of the multiple-discriminant technique. The individual is preserved as a vector of all his characteristics, taken together with all they convey as to size and shape by both absolute magnitude and by covariation. The relative contribution of each variable in discriminating between groups is assessed, and "weights" are assigned accordingly. Therefore, only what is really significant in the particular comparison is utilized, and exactly which variables are important and in what magnitude is brought out by the technique. The technique deals with the possibility of a particular variable, which in itself is not a good discriminator between groups, emerging as highly valuable when taken in combination with other variables.

The amount of calculation involved in this type of analysis has severely limited its use of anthropology until recently; a computer is a necessity. Pearson's "coefficient of racial likeness" or C_2 was one of the earliest statistics to utilize multiple measurements, and was a test of the statistical

significance of difference between populations rather than a true measure of biological distance. This statistic was later revised to be a measure of biological distance, and to handle intercorrelations. Mahalanobis (9) developed a still useful statistic referred to as D^2 , or the generalized distance statistic. The D^2 procedure measures the extent and direction of morphological separation between groups, and makes possible an analysis of relationships between continuous variables, discounting the correlation existing between them. Fisher (7) considered D^2 as the best available statistic for estimating biological similarities and differences between groups. In 1936, the concept of discriminant function analysis was introduced by Fisher, a new method of testing significance for multiple measurements. A procedure was devised for estimating test weights in such a way that a linear combination of the weighted scores (called discriminant function coefficients) would provide a maximum discrimination between groups of individuals. The compounded measurements maximize the variation between groups, relative to the variation within groups. The use of multiple-discriminant analysis in anthropological research is only now becoming common, and is not as yet widespread. With the increasing availability of computer facilities the calculation necessary for its application will become less of a problem. A number of investigators have employed it in research of an anthropological nature (3,8,10). Fortran computer programs for multiple-discriminant analysis are available (5,6). Dixon's BMD 04M program computes means of the variables for each group and the mean difference, variance-covariance matrix, inverse of the variance-covariance matrix, discriminant function coefficients, Mahalanobis' D^2 and associated F statistics, and the discriminant scores for the groups with their means, variances, and standard deviations. Scaled vector values to show the relative contributions of the variables to each function may be obtained from the Fortran V program Multivariate Discriminant Analysis (2). Scaled vector values are discriminant function coefficients computed after standardization of variables, and give an accurate picture of the relative contributions of the particular measurements or indices contributing to discrimination. In applications of the method to hybridization or classificatory studies, a high scaled vector value indicates a major contribution to group separation, or a low relative similarity between groups for the particular variable. A low scaled vector value indicates that the variable does not contribute greatly to group separation, and is a biological feature which has not changed between, for example, a hypothetical ancestral population and a hybridized descendant group. A "morphological score" is computed for each individual hybrid skull on the basis of the discriminant function coefficients obtained from the comparison with potential ancestral or contributor series. The biological position of each individual is assessed, in addition to the relationships between the series considered as units. The relative contributions of each series, with respect to each variable used, is obtained. In classificatory studies, the position of each individual may be assessed and the specific factors accounting for this position identified. An initial set of variables may be reduced to a smaller number of variables for further studies, and the overall direction of morphological trends in the samples obtained.

Since in a multivariate analysis it is possible to treat a skull as a unit, rather than as a series of single traits, and to compare these traits in population terms, inter-related measurements likely to bring out aspects of size and shape characterizing populations should perhaps be the goal in mind. Characteristics which would be useful in characterizing populations may be identified which are not at all those which have been traditionally used in univariate skeletal comparisons. In this regard, there is much need for continued experimentation.

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BOTANY

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WILLARD F. YATES, JR., Department of Botany, Butler University
Indianapolis, Indiana 46208, was elected Chairman for 1972

ABSTRACTS

Inorganic Arsenate as a Tool for Genetic and Biochemical Analysis of Photosynthetic Metabolism in Algae. ROBERT K. TOGASAKI and MARGARET O. HUDOCK, Department of Botany, Indiana University, Bloomington 47401.—Wild type cells of *Chlamydomonas reinhardi* were grown on acetate supplemented agar plates, containing varied concentrations of inorganic arsenate, and the number of surviving colonies were scored. The concentration of arsenate required to inhibit growth was lower in the light than in the dark. Addition of 3-(3,4-dichlorophenyl) 1-1,1-dimethylurea, or DCMU, to the solid medium decreased the cell's sensitivity towards arsenate in the light, but did not affect sensitivity in the dark. Several mutant strains, each carrying a specific defect in its photosynthetic apparatus, were tested for their response towards arsenate, and all were found to be more resistant in the light than wild type cells. The potential application of this system to genetic analysis of photosynthesis, and also some possible explanations for the observed phenomena was discussed.

The "Liquid" Endosperm of Grasses. PAUL WEATHERWAX, Department of Botany, Indiana University, Bloomington 47401.—A few species of Gramineae have an endosperm which remains soft and plastic even after the seeds have been stored dry for many years. The cell walls are thin and fragile, and the stored food consists of what seems to be a fatty matrix surrounding the nucleus and numerous minute starch grains. This type of endosperm is distributed in such an erratic way in the grass family that thus far it seems to have very little taxonomic significance.

The Dichotomous Dilemma. ARTHUR T. GUARD, Department of Biological Sciences, Purdue University, Lafayette, Indiana 47907.—Very early in the history of plant morphology, the growing apex of multicellular plants became recognized as a focal point of interest. The final form of the plant is very largely determined by the development of this apex and the appendages which it produces. The biochemical compounds which so profoundly affect the plant's development often originate in this area.

In view of these facts, it is indeed surprising that one finds such confusing and conflicting usage of the term, "dichotomous branching", in the descriptions of apical growth and development. This paper presented some of the observable forms of apical branching and how the term has been used in reference to these forms.

A Comparison of Modern and Cretaceous *Sassafras* Leaves. RONALD A. RIEPE and DAVID L. DILCHER, Department of Botany, Indiana University, Bloomington 47401.—A study was undertaken to reevaluate the fossil record of *Sassafras* using a comparison of leaf form and fine

venation of modern and fossil material. *Sassafras*, a monotypic genus which has been identified early in the fossil record of angiosperms, has leaves that are entire, asymmetrically bilobed, or trilobed, but may possess up to six lobes (Berry, 1902). The form of the lobes and sinuses may be extremely variable. Berry stated that the venation of the basal portion of the blade was a constant character which could be used to differentiate *Sassafras* from *Aralia*, *Cissites*, and *Platanus*. The distinctive marginal vein pattern of *Sassafras* may also be used in classification, however this character could be obliterated easily or overlooked in fossil forms. Ward (1887) pointed out the remarkable character of the marginal veins of the sinuses in modern leaves. Lesquereaux (1892) wrote of many of his identifications of leaf remains from the Dakota Group with some uncertainty. By 1902, twenty-eight American forms of *Sassafras* had been published. Berry retained only six forms in the genus *Sassafras*, referring others to *Aralia*, *Cissites* and *Platanus*. The leaf form and venation characters of *Sassafras* have been reevaluated and applied to the fossil record.

Structure of Glandular Hairs of Marihuana. CHARLES T. HAMMOND and PAUL G. MAHLBERG, Department of Botany, Indiana University, Bloomington 47401.—Glandular hairs on the bracts of pistillate flowers of marihuana (*Cannabis sativa* L.) were viewed with the aid of light, electron transmission, and electron scanning microscopy. Three morphologically distinct types of glandular hairs occur on bracts: bulbous, capitate-stalked, and capitate-sessile. Bulbous glandular hairs, which are small and composed of only a few cells, consist of a globular head of one to several cells positioned on a basal cell or cells. Capitate glandular hairs, whether stalked or sessile, have a flattened multicellular cap composed of 8 to 13 cells which are covered by an abundant secretory product. The secretory product accumulates beneath a membranous sheath which is derived from the outer surface of the cap cells. Maturing cap cells have a large central vacuole with a very dense peripheral cytoplasm. The cytoplasm contains dictyosomes, endoplasmic reticulum, and plastids with crystalline inclusions. Large pores in the walls between neighboring cells contribute to the formation of a continuous protoplast in the cap. The possible association of capitate glandular hairs with production and/or localization of the hallucinogen tetrahydrocannabinol (THC) was discussed.

Chromic Acid as a Selective Stain for Laticifers in *Vinca rosea*. LARRY R. YODER and PAUL G. MAHLBERG, Department of Botany, Indiana University, Bloomington 47401.—A mixture of equal parts of 10% chromic acid and 10% nitric acid added to living tissues of *Vinca rosea* causes some cells to turn bright red within a few minutes. Acidified chromate and dichromate salts produce the same effect indicating the dichromate anion as the reactant. Reacting cells included occasional parenchyma cells in the pith, stem cortex and root cortex along with laticifers in all parts of the plant. Use of this staining procedure permitted delineation of *Vinca's* entire non-articulated, unbranched laticifer system with a minimum of preparation. Some coloration appears if latex exudes directly into the reagent, but no reaction occurs if latex stands 30 to 60 seconds. Staining only occurs when living cells are treated and the coloration fades after 10 to 15 minutes and cannot be restored. Tissues which have been

frozen, fried, heated above 55 degrees Centigrade, or treated with an fixative are unreactive. The same cells vitally stain with neutral red and toluidine blue indicating a low pH and possible lysosomal activity. Although the reaction is not common to all latex-bearing plants, coloration does appear in laticifers of *Vinco minor*, *Plumeria*, *Asclepias* and some euphorbias. The reaction is postulated to be dependent upon a lower pH in these specialized cells, and any treatment which interferes with maintenance of this acid condition also blocks the reaction with chromic acid. The precise nature of this reaction is yet to be determined.

The Terminal Inflorescence of the Maize Mutant *Tassel seed-2*. C. L. GEHRING, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809, and S. N. POSTLETHWAIT, Department of Biological Sciences, Purdue University, Lafayette, Indiana 47907.—A morphological and histological examination of developmental changes in the terminal inflorescence of the maize mutant, *Tassel seed-2* was conducted. Plants were grown in environmental control chambers under the conditions: 80 degrees Fahrenheit during 16 hours of artificial light (flourescent and incandescent) and 65 degrees during an 8-hour dark period.

Two plants a day were removed from the growth chamber starting 19 days after planting and terminating 67 days after planting. Examination of these plants revealed that the sequence of development and arrangement of floral structures are similar to comparable structures in normal maize. Primordia for both pistillate and staminate flowers are initiated in all flowers. However, in tassels which develop functional pistillate flowers, the staminate primordia abort. Pistillate primordia abort in the tassels which develop functional staminate flowers.

One hundred and nine days after planting, 28 plants were harvested; 16 plants had 100% pistillate tassels and they averaged 560.6 silks per tassel and 12 plants had completely staminate tassels which averaged 281.3 stamens per tassel.

A Review of the Fossil Apocynaceae from the Eocene of Western Tennessee and Kentucky. GARY E. DOLPH, Department of Botany, Bloomington 47401.—Fossil leaves similar to those described by Berry in the form genus *Apocynophyllum* were collected from the Claiborne Formation (Middle Eocene) of western Tennessee and Kentucky. Fossil material representing three of Berry's original eleven species of *Apocynophyllum*—*A. mississippiense*, *A. crassum*, and *A. wilcoxense*—was found. Fossil leaves of *A. mississippiense*, a species characterized megascopically by closely spaced major lateral veins and a broadly alate petiole, were divided into two groups based on the presence or absence of cuticular flanges on the abaxial cuticle. In addition, two previously undescribed leaf types which also possessed alate petioles were distinguished from *A. mississippiense*. The first showed a much greater spacing between the major lateral veins than *A. mississippiense* but yielded no cuticle. The second, whose gross morphological features (except for the alate petiole) were totally distorted, yielded a cuticle which lacked the trichome bases characteristic of *A. mississippiense*. *Apocynophyllum crassum* has a broad petiole that

is woody throughout and not alate as in *A. mississippiense*. In the preceding four leaf types where cuticle has been isolated, the paracytic accessory cell arrangement common to some extant members of the Apocynaceae was found. Although a superficial resemblance exists with regard to accessory cell arrangement, differences between the morphological characteristics of the fossil leaf types and that of the extant members of the Apocynaceae precludes the assignment of the fossil material to any extant genus of the Apocynaceae. Fossil leaves, whose closely spaced, subparallel major lateral veins terminate in a distinct marginal vein, were divided by Berry among *A. wilcoxense* and select fossil species of *Ficus* and *Myrcia*. Upon examination, only one leaf type characterized by internal mucilaginous cavities was discerned. Internal mucilaginous cavities are not characteristic of the Apocynaceae or Moraceae but are found in the Myrtaceae.

Revaluation of *Engelhardia* of the Eocene of Southeastern United States.

FRANK W. POTTER, JR., and DAVID L. DILCHER, Department of Botany, Indiana University, Bloomington 47401.—Several species of fossil fruits, leaves and pollen from the Eocene of southeastern United States have been assigned to the genus *Engelhardia*. Recent examination of modern representatives of Old and New World sections indicates sufficient diversity to warrant segregating the New World members into the genus *Oreomunnea*. Two fruits of *E. puryearensis* Berry were obtained from the Puryear clay pit, Puryear, Tennessee; both specimens are impressions of the three anterior lobes. No fourth posterior lobe is present. The anterior wings of the fossil show venation patterns similar to the New World group, particularly *O. mexicana*. Based on the fruits, *E. puryearensis* is more closely aligned to Central American populations than Asiatic taxa. Other *Engelhardia* fruits reported by Berry suggest the presence of both genera in the southeastern United States during the Eocene. Modern pollen is not sufficiently distinctive to conclusively separate New and Old world sections. To date, no fossil leaves have been obtained that can be assigned to either the genus *Engelhardia* or *Oreomunnea*, although the two extant genera show distinct morphological and cuticular differences. The presence of leaves could be important for determining the relationship of the fruits to *Engelhardia* and/or *Oreomunnea*.

Middle Eocene Sabaloid Palms. C. P. DAGHLIAN and D. L. DILCHER, Department of Botany, Indiana University, Bloomington 47401.—E. W. Berry reported three palm types from the middle Eocene clay deposits of western Kentucky and Tennessee. Two of these were pinnately compound types. The third being palmately compound. The palmate palm fossils in the Indiana Paleobotanical collection from the same sediments suggest that there are more than one genus and species of palmately compound palm leaf fossils. Berry's specimens of *Sabalites grayanus*, were identified primarily on the basis of gross features, leaflet distribution and venation. A detailed study of the palm material from the Indiana University collection is in progress and a thorough examination of fine features, particularly cuticular characteristics, as well as gross features has indicated that there are clearly two and perhaps three kinds of palmately compound palm leaf fossils rather than the one that Berry

reported. The first group has been identified as a member of the genus *Sabal* indicating that it was a clearly defined group as early as the middle Eocene. These palms are large, costa-palmate leaf fragments similar to modern sabals such as *S. texana*. The second group is comprised of palmate leaf fragments of *Sabalites grayanus* but contains two subgroups. Those show differences in venation and the pattern of their stomatal apparatus. These findings are important because they show a greater diversity of palms in the flora of these middle Eocene sediments.

Cell Wall Regeneration Around Protoplasts Isolated from *Convolvulus arvensis* Tissue Culture. RANDALL K. HORINE, Department of Biology, Earlham College, Richmond, Indiana 47374, and ALBERT W. RUESINK, Department of Botany, Indiana, University, Bloomington 47401.—Protoplasts of *Convolvulus arvensis* L. tissue culture regenerated a wall-like structure within 3 days in culture. Although unusually electron dense and atypically amorphous in the electron microscope, this structure could be digested with cellulase but was resistant to protease, pectinase, and β -1,3-glucanase just like the original wall. A cytochemical test for callose was negative. Wall regeneration required a readily metabolized external carbon source and was not inhibited by a high concentration of cycloheximide, puromycin, or actinomycin D, suggesting that stable, long-lived protein or messenger RNA is involved in its synthesis. Protoplast budding was correlated with the wall regeneration and the latter was related quantitatively to the sucrose concentration in the medium. Auxin (2,4-D) neither promoted nor inhibited wall regeneration at any concentration. Culturing protoplasts in the presence of proteolytic enzymes decreased their ability to undergo cell wall regeneration, although the concentration required was so high as to suggest that the proteins involved must be at least partially buried in the plasma membrane.

Initiation of Callus Tissue of *Abies concolor* (White Fir) by Tissue Culture Techniques

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Abstract

Callus tissue of *Abies concolor* (Gord. and Glend.) Lindl. was initiated from inocula prepared from terminal stem tissue on a modified Winton medium containing basis salts, organic nutrients, growth regulators and a stem extract of *Abies concolor*. Under controlled environmental conditions, the callus has grown and has been maintained through subsequent subculture for ten months.

Introduction

Culturing of gymnosperm tissues under aseptic conditions has been difficult to achieve. Approximately 27 gymnosperm species have been successfully cultured, however (1-13). Among these 27 is grand fir (*Abies grandis*) the only representative of the genus *Abies* to have been successfully cultured to date (6).

We now report the initiation of callus tissue from stem tissue of mature *Abies concolor* (Gord. and Glend.) Lindl. trees.

Materials and Methods

The inocula used in this study were obtained from branches (which were less than 3 m above the ground level) of mature *Abies concolor* trees. Terminal stem sections approximately 10 cm long and consisting of the current year's growth were used; before taking, each stem section was sprayed with 70% ethyl alcohol. Cuts were made with sterile scalpels. After making the cut, the basal end of each stem section was sprayed with 70% ethyl alcohol and placed in a separate, unused, plastic bag and the opening of the bag was fastened securely.

In the laboratory, one stem section at a time was removed from its plastic bag and immersed in a shallow container of 70% ethyl alcohol. While immersed in the alcohol, all the needles were removed with a scalpel at their junction with the stem. Each de-needled stem section was transferred to a dry, sterilized, 500 ml Ehrlenmeyer flask equipped with a screw cap. After the stem sections were de-needled and placed in the flask, 250 ml of fresh, household bleach (5.25% sodium hypochlorite) were added, along with 2 drops of Tween 20 (polyoxyethylene sorbitan monolaurate), a wetting agent used as a surfacant. The flask was agitated frequently during the 10-minute interval that the stem sections were sterilized. The bleach was decanted, and the stem sections were rinsed 3 times with sterile, distilled water. The sterilized portions of stems were cut into sections approximately 8-10 mm in length. The end sections were discarded, while the others were inserted, basal end up, in 25 ml of an agar nutrient medium in 20 x 100 mm petri dishes.

Results and Conclusions

Callus was successfully initiated and maintained on a modification of Winton's medium for triploid quaking aspen (14-17). Winton's medium was modified by the addition of gibberellic acid, indoleacetic acid, naphthaleneacetic acid, ascorbic acid, L-glutamine, myostatin (a fungicide), and a stem extract of *Abies concolor*. On this undefined medium (Table 1), cell proliferation occurred within 14 days while the plates were being maintained in the dark in an incubator at $27.5 \pm 2^\circ \text{C}$ under satur-

TABLE 1. Undefined medium for initiating and maintaining *Abies concolor* callus.

Constituents	Conc mg/l	Constituents	Conc mg/l
MgSO ₄ ·7H ₂ O	764.0	pyridoxine	0.1
Na ₂ SO ₄	425.0	Fe-EDTA	5.5
Ca(NO ₃) ₂	170.0	2,4D	0.5
KNO ₃	425.0	kinetin	1.0
KCl	140.0	sucrose	20000.0
NaH ₂ PO ₄ ·H ₂ O	34.0	bacto-agar	8000.0
myo-inositol	100.0	gibberellic acid	0.5
MnSO ₄	9.0	indoleacetic acid	1.0
ZnSO ₄ ·7H ₂ O	3.2	naphthaleneacetic acid	1.0
H ₃ BO ₃	3.2	ascorbic acid	0.1
KI	1.6	L-glutamine	250.0
nicotinic acid	0.5	myostatin	2.5 ¹
thiamin	0.1	<i>Abies concolor</i> stem extract	85.0 ^{1,2,3}

¹ml/l

²The stem extract was prepared by adding 150 g of terminal stem sections of the current year's growth of *Abies concolor* to 50 ml of distilled water; pulverizing for 15 min at high speed in a Waring Blender; and suction filtering and collecting the liquid extract.

³pH of medium was adjusted to 5.7 with 0.1N NH₄OH.

ated humidity conditions. Figure 1 shows the small mound of callus which formed on representative inocula after 42 days of growth. At 42 days the callus tissue was transferred to fresh medium where it continued to grow and, thereafter, was subcultured every 21 days. Figure 2 shows a representative mound of callus tissue after 90 days. Cells on the surface of tissue this age are still near white, but basal cells are reddish-brown. The pigmentation in the older cells did not appear to hinder the proliferation of *Abies concolor* callus since the callus has been subcultured and maintained for 10 months. Harvey and Grasham (6) also reported pigmentation

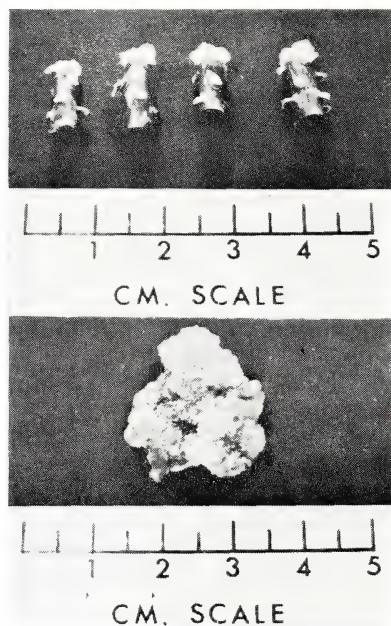


FIGURE 1. *Abies concolor* callus at 42 days (top).

FIGURE 2. *Abies concolor* callus at 90 days. (bottom)

in the callus of *Abies grandis*, but it lost its vigor by the end of 90 days. Further study with the established *Abies concolor* callus is now in progress; parameters being investigated are growth rate, minimal nutrient medium, effects of varying photoperiod, and callus differentiation and maturation.

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CELL BIOLOGY

Chairman: WILLIAM J. GRIFFIN, Eli Lilly and Company,
Indianapolis, Indiana.

CHARLES W. GOFF, Life Sciences Department, Indiana State
University, Terre Haute, Indiana 47809, was elected Chairman
for 1972

ABSTRACTS

Electron Microscopic Study of *Anaplasma marginale* by Negative Staining.

F. PADGETT and W. A. SUMMERS, Department of Microbiology, Indiana University School of Medicine, Indianapolis 46207.—Studies of the morphologic features and reproduction of the initial bodies of *Anaplasma marginale* were done by the negative contrast (phosphotungstic acid) technique of unfixed, dehemoglobinized, infected bovine erythrocytes. The internal structure appeared to be organized as a coiled, tubular, or ribbon-like structure. The reproduction process, reconstructed from observation of static preparations, began with the formation of a "budlike" structure in the initial body.

Growth Cycle of *Penicillium chrysogenum* Virus. L. F. ELLIS and R. J. DOUTHART, Eli Lilly and Company, Indianapolis, Indiana 46206.—

Penicillium chrysogenum was grown in stainless steel fermenters with continuous agitation and aeration. During the fermentation aliquots were withdrawn and were centrifuged to harvest the cells. Either the virus particles of the double stranded RNA (dsRNA) was isolated from these cells. The amount of dsRNA per gram of mycelia increases until approximately 60 hours incubation and then declines. Physical and biological properties suggest this dsRNA is of viral origin. Other aspects of this virus were discussed.

Toxic Response of Mice to a DDT Diet, Its Suppression by Feeding and the Effect of Estrogen. DOROTHY A. WERDERITSH, D. SHELTON, C. SWAN, C.-M. HAUN, W. YUNGHANS and D. J. MORRE, Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana 47907.

—Mice, 4 to 5 weeks old, of the C3H/HeJ strain or from the Purdue University colony received *ad libitum* water and laboratory chow to which corn oil (1:10, weight/weight) was added. For those animals receiving DDT, technical grade DDT (1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl) ethane) was added to the corn oil to give a final concentration of 900 parts per million DDT in the total diet. Overt signs of DDT toxicity appeared as early as the 5th day after DDT feed was offered. Most of the animals died within 3 days after the onset of severe symptom development. If the animals were withdrawn from the DDT feed and presented with control feed in the initial stages of tremor development, they recovered within 8 to 12 hours and exhibited normal behavior. Yet, if animals previously receiving DDT feed were fasted to starvation levels several weeks later, symptoms of DDT toxicity reappeared. Control animals fasted to the same weight levels did not develop tremors or other overt signs of DDT toxicity. It was not possible to prevent, reverse or significantly delay

the toxic response to DDT by administration of estrogen (subcutaneous injection of 5 micrograms estradiol cyclopentylpropionate in 100 microliters corn oil per animal). Apparently, a significant factor in effecting recovery of the animals from DDT toxicity is the removal of the source of the DDT and offering of a normal diet to reestablish a pattern of weight gain and caloric balance.

NADH Dehydrogenase Activity of Golgi Apparatus from Rat Liver.

C. M. HUANG, D. J. MORRE, Department of Botany and Plant Pathology, and T. W. KEENAN, Department of Animal Sciences, Purdue University, Lafayette, Indiana 47907.—Highly purified rat liver Golgi apparatus (GA) fractions exhibited NADH dehydrogenase of specific activity approximately one-fourth that observed with endoplasmic reticulum (ER). With exogenous cytochrome *c* as electron acceptor, apparent K_m 's for NADH were 6.17 and 6.09 μM for GA and ER, respectively. The activity in both membrane fractions was insensitive to rotenone and Pieridine A, potent inhibitors of mitochondrial NADH dehydrogenase. With ferricyanide as acceptor, the apparent K_m for ferricyanide was 26.7 μM for both ER and GA and excess of substrate inhibited activity with both membrane fractions. Maximal velocities for the NADH-ferricyanide reductase were 3.44 and 1.44 μM NADH oxidized/min/mg protein for ER and GA, respectively.

The high degree of similarity of these activities comparing ER and GA suggest that they are catalyzed by the same enzyme present in both membrane fractions. The results support the hypothesis that membrane material derived directly from ER is utilized for formation of GA cisterna.

Golgi Apparatus-Medicated Cytomembrane Differentiation in Rat and Bovine Mammary Gland.

T. W. KEENAN, Department of Animal Sciences, and C. M. HUANG and D. J. MORRE, Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana 47907.—Golgi apparatus (GA), endoplasmic reticulum (ER) and plasma membrane (PM) fractions were isolated in highly purified form from both rat and bovine mammary glands and subjected to compositional analysis. Included in the analysis was the milk fat globule membrane (GM), a membrane which is derived directly from the secretory cell plasma membrane. With regard to lipid composition, GA was intermediate between ER at one extreme and the PM and GM at the other with regard to levels of total lipid phosphorus, phosphatidyl choline, sphingomyelin, cerebiosides and cholesterol. Fatty acid composition of GA polar lipids was intermediate between ER and PM. Polar lipids of ER contained higher levels of unsaturated fatty acids whereas PM and GM contained higher levels of saturated fatty acids.

Levels of membrane protein-bound sialic acid were lowest in ER, intermediate in GA and highest in PM and GM. Salt-washed ER, GA, PM and GM membranes displayed several protein bands with identical electrophoretic mobilities. Golgi apparatus was also intermediate between ER and the PM and GM with respect to specific activities of the enzymes NADH dehydrogenase, 5'-nucleotidase, and adenosine triphosphatase.

These results are compatible with the hypothesis of GA-mediated cytomembrane differentiation. Further, they confirm previous results obtained with rat liver cytomembranes and extend them to another cell type with different secretory functions.

Ultrastructure of Green Plastids in Leaves of Genetic Albino Tobacco.

ANNE A. SUSALLA and PAUL G. MAHLBERG, Department of Botany, Indiana University, Bloomington, Indiana 47401.—Genetic green and albino tobacco seedlings were grown on agar with defined nutrients, growth substances and light conditions. Albino plants developed patches of green on the first and succeeding leaves even though the cotyledons were always white. In later stages of growth, these plants became highly variegated with some approaching the phenotype of normal green plants. In ultrastructural studies, some mutant-green plastids of the albino plants have grana that are massive in size with many appressed thylakoids and irregular margins. Other plastids are large with tapered margins to give a spindle effect while a few have only scattered thylakoids. Osmiophilic globules and DNA-like fibrils are present in the stroma. Starch is evident but photosynthetic activity is yet to be determined.

Use of the Scanning Electron Microscope in the Investigation of Cardiac and Pulmonary Tissue.

SHIRLEY SIEW, Indiana University School of Medicine, Indianapolis 46202.—Scanning electron microscopic studies were performed on both fresh biopsy and autopsy specimens of cardiac and pulmonary tissue. The fresh material was obtained at open heart surgery and was fixed in Muir's paraformaldehyde electron microscopy fixative. After fixation, it was processed by three different methods for scanning electron microscopy:

- 1) placed in glycerine overnight
- 2) post-fixed in osmic acid and then placed in propylene glycol
- 3) treated with acetone

The specimens were then coated with gold under vacuum and were examined in a Cambridge Stereoscan Microscope. Chronic rheumatic aortic and mitral valves, papillary muscle and a biopsy of the left lingula were examined. The autopsy material comprised cardiac valves and lung. The latter was fixed under inflation. The best results were obtained in studies of valvular and pulmonary tissue by means of the second method. It was concluded that the scanning electron microscope will prove of value in the investigation of valvular and pulmonary disease.

Ultrastructure of Mouse Heart Cell Culture.

G. B. BODER and L. F. ELLIS, The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206.—Newborn mouse ventricles were dispersed by short treatment with collagenase. Cultures derived by this method have a high proportion of beating cells which attach to collagen, glass or plastic within a few hours of isolation. Beating cells have been maintained in sterile culture in the absence of antibiotics for as long as 6 months and remain responsive to hormones. A photoelectric recording system was developed which is capable of measuring the rate and amplitude of contractions of a single myocardial cell or a colony of myocardial cells. The effects of

drugs on these cells was reported in 1971 by Boder *et al.* (Nature 231: 531-532). We have been studying the ultrastructure of the cultured cells.

This report compared the cultured cells to those of the intact myocardial tissue. Myofibrillar filaments were observed in the cell cultures.

Ultrastructures of Neuronal Lipofuscin and Ceroid. ITARU WATANABE, VIMAL PATEL, and WOLFGANG ZEMAN¹, Department of Pathology, Indiana University School of Medicine, Indianapolis 46202.—Lipofuscin, an autofluorescent yellow pigment in the residual body, is considered to be indigestible by lysosomal acid hydrolases. This pigment is stained positively by conventional fat staining technique and hence the term "lipid pigment". The pigment is also called "wear and tear" or "age" pigment because it is found most abundantly in the cells of aged individuals. Actually, in the senile brain, the material occupies a large area of the nerve cell body by displacing the nucleus and other cytoplasmic organelles. Electron microscopy revealed that the lipofuscin bodies are surrounded by trilaminar limiting membrane and contain fine granular osmiophilic material. Using histochemical techniques for electron microscopy, we observed acid phosphatase activity in the granular matrix but not in the lipid portion. Our findings agree with observations by others. Recently, we also demonstrated some evidence of peroxidation in the lipid portion of this organelle, supporting the view that lipopigment is derived by peroxidation of fatty acids and proteins.

Another pigment called "ceroid" is found to accumulate in brain cells of patients with Neuronal ceroid lipofuscinosis. Ultrastructural aspects of these two types of pigments, lipofuscin and ceroid, in the human brain were presented.

¹The authors acknowledge the technical assistance of Vera Kolar, Janice Herring and Connie Aryea.

Effect of Beta-alanine on Glucose Catabolism and Growth of Ehrlich Ascites Tumor Cells. M. E. JACOBS, Department of Biology, Goshen College, Goshen, Indiana 46526.—Intraperitoneal injection of 0.1 ml 5 M beta-alanine at the time of infection, and daily thereafter, inhibits tumor-weight gain of Carworth female CF1 mice by 19% by the end of 14 days, as compared with infected mice injected with 0.1 ml 2.5 M NaCl. Incubation of the tumor cells at 37.5°C in 25 mM beta-alanine in Krebs-Ringers bicarbonate solution at pH 7.4 inhibits ¹⁴CO₂ excretion from the cells using 1-¹⁴C-D-glucose as substrate. The inhibition is 18-25%, as compared with control samples containing no beta-alanine, or 25 mM of beta-aminoisobutyric or gamma-aminobutyric acids.

NOTE

Inhibition of Succinate Oxidation in Beef Heart Mitochondria by Derivatives of Pyridine Adenine Dinucleotide. P. V. BLAIR and L. Y. CHAO, Indiana University Medical Center, Indianapolis, 46202.—Succinate oxidation is inhibited by nicotinamide adenine dinucleotide (NAD⁺) in damaged or sonicated mitochondria. Two mechanisms to account for this NAD⁺-inhibition have been suggested. In 1948, Pardee and Potter (1) postulated that inhibition occurred because NAD⁺

stimulated the production of oxaloacetate an inhibitor of succinate oxidase, from succinate *via* fumarate and L-malate. In 1963 an alternative hypothesis was proposed by Neubert *et al.* (2) in which binding of NAD⁺ or one of its analogues to a site on succinate dehydrogenase would cause direct inhibition of succinate oxidation. Experiments to determine the most likely explanation for NAD⁺-inhibition of succinate oxidation were carried out on preparations of beef heart mitochondria.

All dinucleotide tested, NAD⁺, 3-acetylpyridine adenine dinucleotide (3-AcPAD⁺), 3-acetylpyridine deaminoadenine dinucleotide (3-AcPDeAD⁺), nicotinamide adenine dinucleotide phosphate (NADP⁺), NADH and NADPH with the exception of NADPH inhibited succinate oxidation. The inhibition had a lag period and increased with time in the presence of all inhibitory dinucleotides except NADP⁺. The increase in inhibition with time by NADP⁺ comes to an early steady (per cent inhibition does not change), probably because it is not a good coenzyme for malate dehydrogenase and because the reduced form (NADPH) can not be oxidized by the electron transfer chain. L-malate, which stimulates the production of oxaloacetate in the presence of NAD⁺ and its analogues, enhanced inhibition and compounds (glutamate, rotenone, NADH *plus* rotenone, and meso-tartrate) which remove oxaloacetate or stop its formation prevent or reverse NAD⁺-inhibition of succinate oxidation. The oxidized dinucleotides do not inhibit succinate oxidation in purified electron transfer particles. A supernatant fraction containing fumarase and malate dehydrogenase restores the inhibitory ability of NAD⁺ to these purified electron transfer particles. The addition of oxaloacetate to these purified particles inhibits succinate oxidation with apparent competitive kinetics.

Differential spectra and oxidation-reduction of the dinucleotides at absorption maxima were recorded in the presence of succinate and L-malate. The appearance of the reduced form of the NAD⁺-analogues was positively correlated with the per cent inhibition of succinate oxidation. The reduced analogues accumulated from malate dehydrogenation because they are not rapidly oxidized by the respiratory chain, whereas NADH does not accumulate because it is rapidly reoxidized by electron transfer. Our results are consistent with the postulate that oxaloacetate is the inhibitor of succinate oxidation and NAD⁺ only stimulates the accumulation of oxaloacetate. None of our evidence would indicate that NAD⁺ directly inhibits succinate dehydrogenation or the succinate dehydrogenase enzyme.

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Scale Calcification In A Chrysophycean Alga: A Test System For The Effects of DDT On Biological Calcification¹

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Abstract

The chrysophycean alga, *Coccolithus huxleyi*, responds to the chlorinated hydrocarbon, DDT, by reduced calcification of surface scales. Cytological and biochemical findings show reduced calcium carbonate deposition on the scales and reduced calcium utilization by the organism.

Chlorinated hydrocarbons [particularly certain types of pesticides such as dieldrin, DDT, DDE (a stable metabolite of DDT) and the polychlorinated biphenyls (PCB's) used as plasticizers]³ at high levels in the diet, result in changes in calcium metabolism which may lead to eggshell thinning and reproductive failure in predators and raptorial birds near the top of the food chain (2, 5-13, 15, 16, 17, 19-21, 24). The mechanism by which these compounds reduce calcium carbonate deposition has not been established. One possibility is that carbonic anhydrase is inhibited (1, 16). This enzyme catalyzes the conversion of carbon dioxide to bicarbonate which then reacts with calcium to form calcium carbonate. Another possibility, frequently mentioned, is hormonal imbalance (1, 10-13, 15-17). Estrogen, for example influences calcium mobilization to the shell gland during eggshell formation. Because of the hormonal complexity of the avian calcification system, simpler systems were sought which might serve as models for the study of DDT effects on eggshell calcification.

The marine alga, *Coccolithus huxleyi*, secretes cell wall scales, certain of which are layered with calcium carbonate (14). The scales are produced within cisternae of the Golgi apparatus and transported to the cell surface via secretory vesicles (14). Calcification occurs during secretion. The purpose of this study was to determine whether DDT influenced calcification in the algal system.

Materials and Methods

Cultures of *Coccolithus huxleyi* (Lohm.) Kamptner were obtained from the Indiana University culture collection. Cells were grown on

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²Recipient of a summer research fellowship sponsored by an award from the Research Grants Committee of the Indiana Academy of Science.

³DDT - 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane

DDE - 1,1-dichloro-2,2-bis (p-chlorophenyl) ethane

Dieldrin - 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*exo*-1,4-endo-5,8-dimethanonaphthalene.

Erdschreiber Enrichment Medium (18), with and without technical grade DDT in a saturated solution, using acetone at a final concentration of 1% as a carrier. Control cells were grown in the presence of 1% acetone. Cultures were maintained under 100 ft-c at 23° C with a 16-hour photoperiod.

Cells were prepared for electron microscopy by fixation in 2% glutaraldehyde in 0.1 M potassium phosphate, pH 7.2, for 1 hour, post-fixation in 1% osmium tetroxide in 0.1 M potassium phosphate, pH 7.2, for 1 hour and dehydration through an acetone series. The tissue was embedded in Epon (23), sectioned and then observed with a Philips EM 300.

Carbonic anhydrase assays followed the procedure of Roughton and Booth (22). Calcium utilization was determined by adding 100 μ C⁴⁵Ca to 3 ml aliquots of cell suspensions grown in the presence and absence of DDT. After 2 hours, the cells were transferred to a solution containing unlabeled calcium for 30 min to remove exchangeably bound

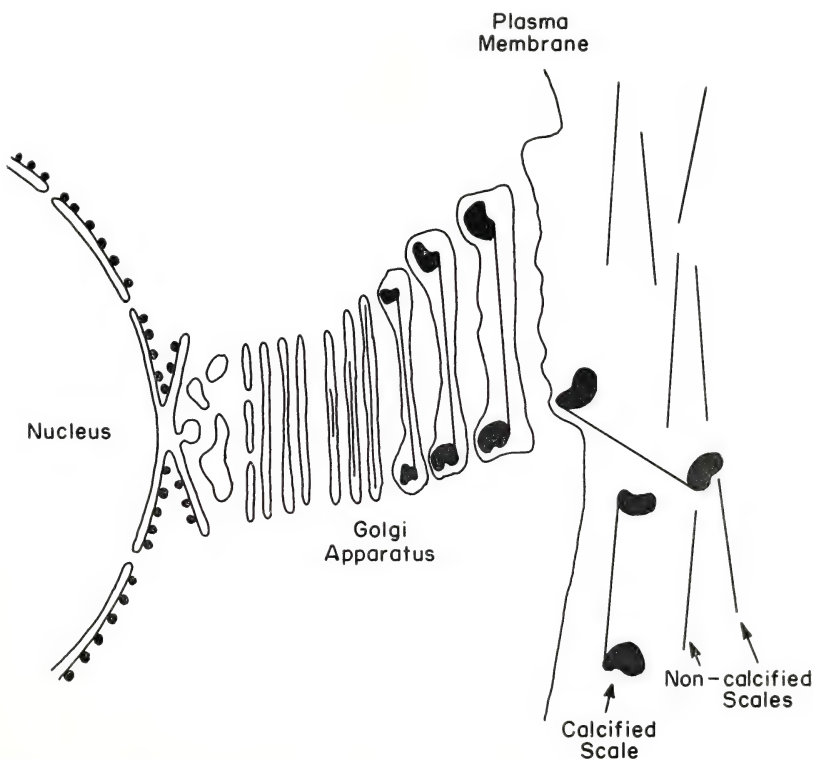


FIGURE 1. Schematic diagram illustrating the process of scale formation in a chrysophycean alga. The scales are formed within cisternae of the Golgi apparatus. The cisternae of the Golgi apparatus separate from the stack and the cisternal membranes fuse with the plasma membrane during discharge of individual scales. Calcification occurs at the scale margin (solid black projections) prior to discharge of the scale to the cell surface. Adapted from Brown et al. (4).

radioactivity. Radioactivity was measured with a Nuclear Chicago gas-flow monitoring system.

Results and Discussion

Coccolithus huxleyi secretes cellulosic scales into the cell wall (14). As with other Chrysophycean algae (3, 4), these scales are formed in the cisternae of the Golgi apparatus and are transported to the plasma membrane in secretory vesicles (14, Fig. 1). The scales are of two types that are secreted in alternating waves. Type I scales are small and non-calcified (Fig. 2A). Type II scales are larger and have a mineralized rim around their edges (Fig. 2B). Calcification takes place while the Type II scales are en route to the cell wall in the secretory vesicles.

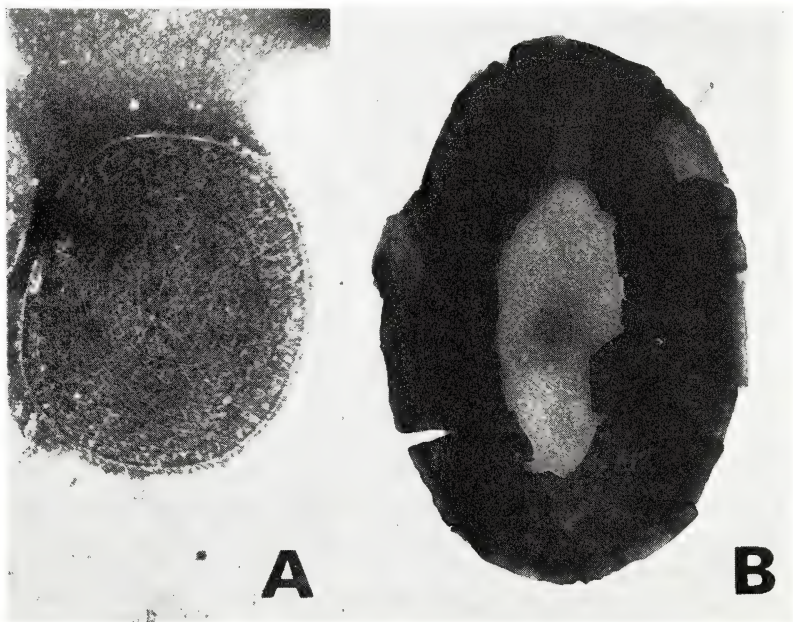


FIGURE 2. Electron micrographs of isolated scales of *Coccolithus huxleyi* after negative staining with 1% potassium phosphotungstate, pH. 6.5 A. Noncalcified (Type I) scale. B. Calcified (Type II) scale. The calcified rim appears in B as a wide electron dense margin at the periphery of the scale. X 33,000.

A typical control cell is shown in Figure 3. The alternating waves of Type I and Type II scales are apparent within the cell. Where calcification is heavy on the Type II scales (scales normally calcified), the mineralized deposit is lost during specimen preparation and only a hole is left. Loss of the deposits from the section is a criterion for heavy calcification that was previously reported by Manton and Leedale (14).

When cells were grown in the presence of DDT, calcification in the wave of Type II scales being formed was markedly reduced (Fig. 4),

whereas the previous wave of Type II scales was normally calcified. Figure 5 shows that the scale and the matrix upon which the

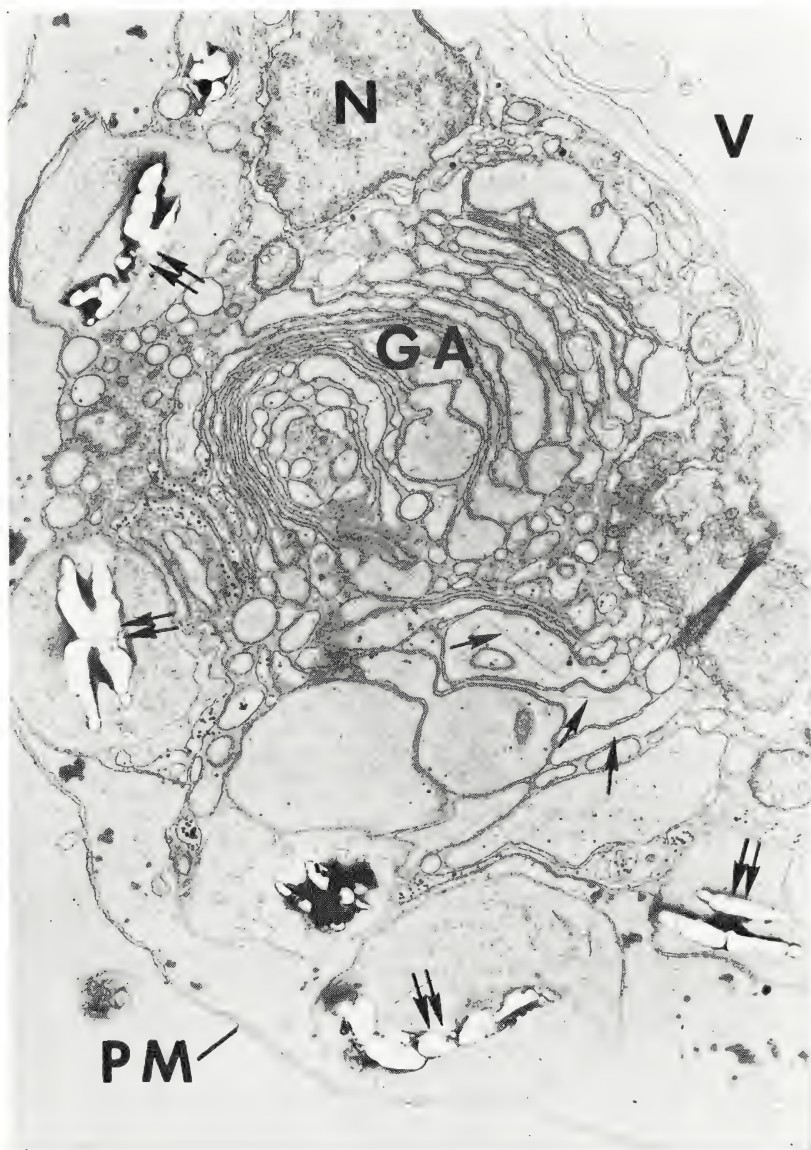


FIGURE 3. Thin section of a control cell of *Coccolithus huxleyi*. Glutaraldehyde-osmium tetroxide fixation. Section post-stained with lead citrate. The outer wave of Type II scales (double arrows) emanating from the Golgi apparatus (GA) is heavily calcified as evidenced by the holes (double arrows) where the heavily mineralized deposits have dropped out during specimen preparation. The inner wave is of the Type I, non-calcified scales (single arrows). PM = plasma membrane. V = vacuole. N = nucleus.

X 26,000.

calcium carbonate is layered are formed normally in the DDT-treated cells, but the amount of calcium carbonate deposited is less than that for control cells.



FIGURE 4. Thin section of a DDT-grown cell of *Coccolithus huxleyi* prepared for electron microscopy as in Figure 3. The outer layer of calcified, Type II scales (double arrows) shows evidence of reduced calcification (compare with Figure 3), but the reduction in calcification is most marked in the inner wave of Type II scales (those still associated with Golgi apparatus cisternae within the cytoplasm; arrows with asterisks). Noncalcified, Type I scales (single arrows) appear normal. PM = plasma membrane. N = nucleus. X 16,000.

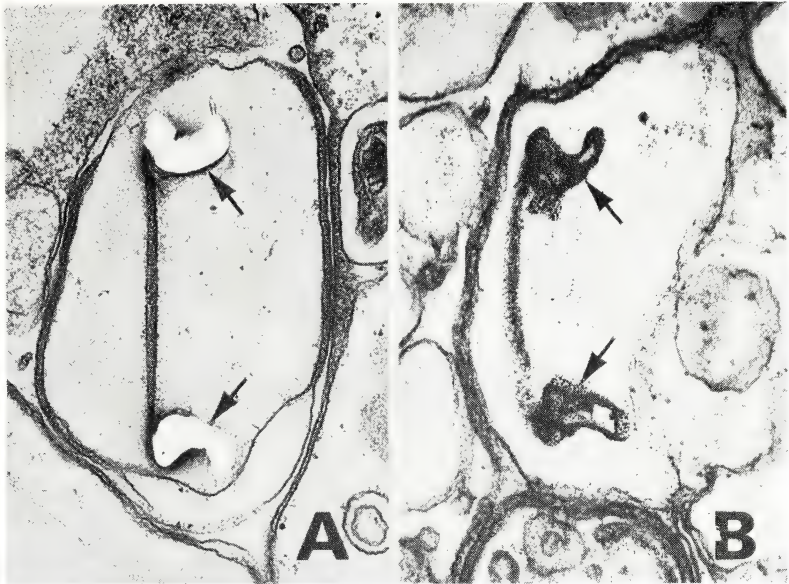


FIGURE 5. Comparison of scales from control (A) and DDT-treated (B) cells of *Coccolithus huxleyi* as seen in thin section. The scale from the DDT-treated cell (B) has developed normally but calcification is reduced (arrows) as evidenced by the absence of the hole-forming mineralized deposits (see Fig. 3 and text) characteristic of control scales (A; arrows). X 40,000.

Further evidence for a DDT effect on algal calcification was sought from determinations of carbonic anhydrase activity. Results summarized in Table 1 show inhibition of carbonic anhydrase from the alga by DDT, although the effect was much less dramatic than expected. The levels of DDT required to inhibit carbonic anhydrase seem high but concentrations of 2,500 ppm of DDT have been reported from thin-shelled eggs of fish-eating birds (16). In experiments with ^{45}Ca , calcium utilization was inhibited by 10 to 15% in the DDT-grown cells.

TABLE 1. Effect of DDT on carbonic anhydrase from *Coccolithus huxleyi*¹.

Extract From	DDT in Assay	Carbonic Anhydrase (Relative Specific Activity)
Experiment I		
Control Cells	None	0.45
	1000 μg	0.36
Experiment II		
Control Cells	None	0.22
DDT-Grown Cells	None	0.12

¹Based on time required to lower the pH of veronal buffer from pH 8.15 to pH 6.30 (22).

The results show that DDT retards the calcification of scales in the alga *C. huxleyi* and that the organism merits further study as a test

system for DDT effects on biological calcification. The alga offers the advantage of a reproducible and inexpensive laboratory system that is free from the complicating hormonal influences found in birds.

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The Effect of Mineral Deficiency on the Photosynthetic Apparatus in Maize. I. The Role of Chloroplast Sulfolipid¹

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Abstract

Galacto- and sulfolipid content has been determined in sulfur and nitrogen-deficient maize leaves. Only slight variations in monogalactosyl diglyceride and digalactosyl diglyceride levels were detected in S-deficient plants compared to normal maize leaves but there was a 50% decrease of both these lipids in N-deficient plants. The greatest difference was found in the sulfolipid content in either S- or N-deficiencies—the sulfolipid was reduced by 30 to 50%. Since decreased sulfolipid content seemed to be associated with increased Photosystem II activity and the occurrence of larger chloroplast grana stacks, it is proposed that sulfolipid may play a role in grana stacking along with other structural chloroplast lipids. At least a correlation between decreased sulfolipid and galactolipid content is associated with the occurrence of larger chloroplast grana stacks and increased Photosystem II activity.

Introduction

In our studies of photosynthetic activity of chloroplasts from nitrogen-, potassium-, phosphorus-, sulfur-, magnesium-, and calcium-deficient plants we found an increased Photosystem II activity in the chloroplasts from S- and N-deficient plants (3). Since increased rates for PS II activity were observed in 2 separate assay systems (water → indophenol dye and diphenylcarbazide → indophenol dye) and were correlated with increased grana stacking in studies of the fine structure of mineral-deficient chloroplasts by electron microscopy (9), it became important to investigate the role of sulfolipids in S- and N-deficient chloroplasts in relation to normal maize sulfolipid content.

Nichols and James (13) have reviewed the literature on chloroplast lipid composition while Haines has surveyed the chemical information on sulfolipids (8). Plant sulfolipids have been localized in the chloroplast and assayed in several species of plants (1, 2, 6, 14, 16). Previous studies on the composition of chloroplast lipids consider sulfolipid a minor constituent (<10% present) and assign a structural role to it. Our discovery of reduced sulfolipid content in S- and N-deficient plants both of which exhibit higher than normal PS II activity points to the importance of sulfolipid not only as a passive structural element but also as something critical for proper enzymatic activity of chloroplast grana stacks.

¹Supported by N.S.F. Grant #GB-27501.

Materials and Methods

Methods for nutrient culture of various mineral-deficient maize plants have been described (5). The galacto- and sulfolipid content of S- and N-deficient plants was determined as follows: 5-g quantities of fresh or frozen maize leaves were homogenized in 90% acetone in a Waring blender and strained through a sintered glass filter. The residue was rewashed with several aliquots of acetone. Then the total acetone extract was transferred to a separatory funnel containing an equal amount of petroleum ether (B.P. 40-60°C). Water-soluble components were removed by water washes and discarded. The green epiphase containing pigments and lipids was washed several times with increasing concentrations of methanol-water (50:50; 75:25; 90:10). The combined methanol-water extracts were evaporated to dryness in a rotary evaporator at 30°C to yield a total lipid fraction. Evaporation was slow and required the addition of several portions of acetone. The final dry lipid mixture was suspended in diethyl ether and used for thin layer chromatography. Silica gel G plates were developed in water, glacial acetic acid, methanol and chloroform (4:10:15:75) according to Nichols (12) or in water, benzene, acetone (8:30:91) by Pohl, Glasl and Wagner's method (15). Monogalactosyl diglyceride, digalactosyl diglyceride and sulfolipid were identified by spraying with 0.0035% rhodamine G in 0.5% KOH solution and observing fluorescent bands under U.V. light. After scraping the desired bands the various lipids were eluted from the silica gel with diethyl ether and evaporated to dryness under vacuum from an aspirator. If plates were developed in mixtures containing glacial acetic acid as in (12), the lipid bands were located by spraying one side of the chromatogram with reduced methylene blue spray according to Barr and Crane (4).

Monogalactosyl diglyceride, digalactosyl diglyceride and sulfolipid were assayed quantitatively by the phenol-sulfuric acid method as described by Roughan and Batt (16). Sulfolipid was also determined as inorganic sulfate after charring and acid hydrolysis in sealed glass tubes. The spectrophotometric analysis of sulfate with barium chloranilate reagent was performed according to Dittmer and Wells (7).

Results

The visible symptoms of sulfur- and nitrogen-deficient maize leaves compared to normal maize are shown in Figure 1 (A). This figure also shows a thin layer chromatogram (B) where the sulfolipid spot is visible as a light charred area in the control but not at all in S-deficient plants in which the sulfolipid content is reduced by about half. Figure 1 (C) shows the results of the phenol-sulfuric acid test for sugars by which monogalactosyl diglyceride and digalactosyl diglyceride can be identified by their galactose moiety and sulfolipid by its quinovose moiety. By using spectrophotometric comparisons to galactose standards, the sulfolipid content of normal maize leaves is higher than that of S-deficient leaves.

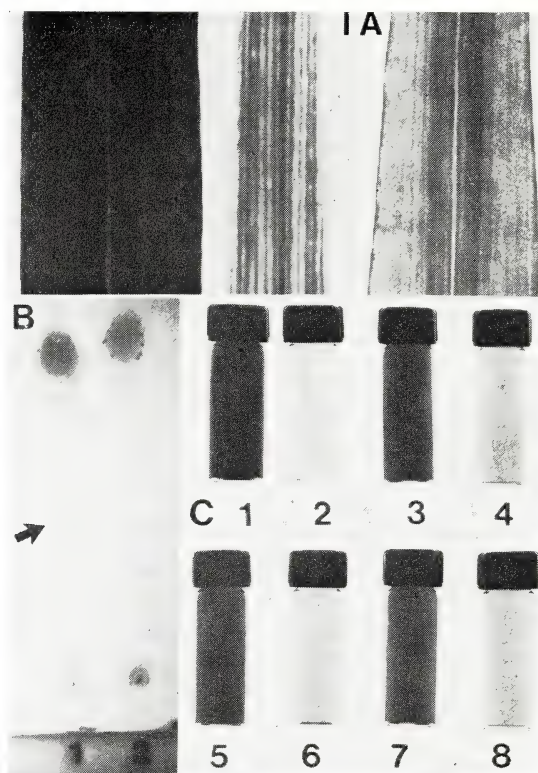


FIGURE 1. A. Control, S-deficient and N-deficient maize leaves from left to right in the order indicated. B. Thin-layer chromatography of sulfolipid, (arrow) from 1) control and 2) S-deficient maize leaves (level below limit of detection by TLC). C. Results of phenol-sulfuric acid test with control and S-deficient maize leaves: 1-4, control; 1-MGD, 2-plate blank, 3-DGD, 4-sulfolipid; 5-8, S-deficient; 5-MGD, 6-plate blank, 7-DGD, 8-sulfolipid.

TABLE 1. A comparison of galactolipid and sulfolipid levels in normal versus S-deficient maize leaves.¹

Designation	By Sugar Analysis		
	MGD	DGD	SL
	$\mu\text{moles/g}$ Fresh Weight		
Control	2.95	1.11	0.85
-S	2.23	1.41	0.35
-N	0.96	0.30	0.48
Designation	By Sulfur Analysis		
	MGD	DGD	SL
	$\mu\text{moles/g}$ Fresh Weight		
Control	—	—	0.84
-S	—	—	0.50

¹Plants grown in nutrient culture in the greenhouse during July and August at high temperatures (25-30°C).

Table 1 summarizes the results of chloroplast lipid analysis. In S- and N-deficient leaves the sulfolipid content is reported from either of 2 separate assays: 1) by analysis of the sugar moiety using the phenol-sulfuric acid test and 2) by sulfur analysis with the barium chloranilate test for inorganic sulfur. The results of both tests indicate a reduced sulfolipid content of S- and N-deficient leaves in contrast to normal maize while differences in galactolipid—monogalactosyl diglyceride and digalactosyl diglyceride—levels are not marked for S-deficient plants but are sharply reduced in N-deficient plants in relation to the control.

Figures 2 and 3 show electron micrographs of normal and S- or N-deficient chloroplasts at the age of 8 weeks. It can be seen that both deficiencies result in larger grana stacks and less stroma lamellae than normal maize plants. The size of osmiophilic globules is increased in chloroplasts from N-deficient plants (Fig. 3, B).

Discussion

From our studies of PS I and II activities in six types of mineral deficiencies (N, P, K, S, Mg, Ca) in maize, we find increased PS II activity in S- and N-deficiencies. Sulphur- and N-deficient plants contain chloroplasts with larger grana stacks and a proportionally larger amount of grana lamelle in relation to stroma lamellae (3, 9). This led us to investigate the lipid composition of S- and N-deficient plants reported here (Table 1), since chloroplast lipids have been implicated as structural components of chloroplast membrane systems. As this table shows we found only slight differences in the two major chloroplast lipids, monogalactosyl diglyceride and digalactosyl diglyceride, in S-deficient versus normal maize leaves but a striking reduction in the amount of galactolipids present in N-deficient plants. The sulfolipid content was also reduced up to 50% in the leaves from S- and N-deficient plants. Thus sulfolipid, although present in lesser amounts than chloroplast galactolipids, may function in the control of grana stacking. Since only about a third to a half of the normal amount of sulfolipid is present in S- and N-deficient plants while the proportion of grana to stroma lamellae is increased, it may be inferred that in the absence of the normal amount of sulfolipid stroma lamellae stick together more easily to form abnormally large grana stacks. Thus sulfolipid appears to control grana stacking in S- and N-deficient maize chloroplasts.

Other agents which alter the control of grana stacking include light and the concentration of cations. In bright light grana stacks are dissociated in *Amaranthus* chloroplasts while in plants grown in dim light abundant grana stacking is present (10). In isolated chloroplasts grana can be dissociated into single lamellae but with the addition of cations reassociation occurs (11). Both of these factors may operate in conjunction with the lipid composition in controlling grana stack formation.



FIGURE 2. *Normal maize chloroplast at the age of 8 weeks. Glutaraldehyde and OsO_4 fixation with uranyl acetate and lead citrate stains. X 25,000*

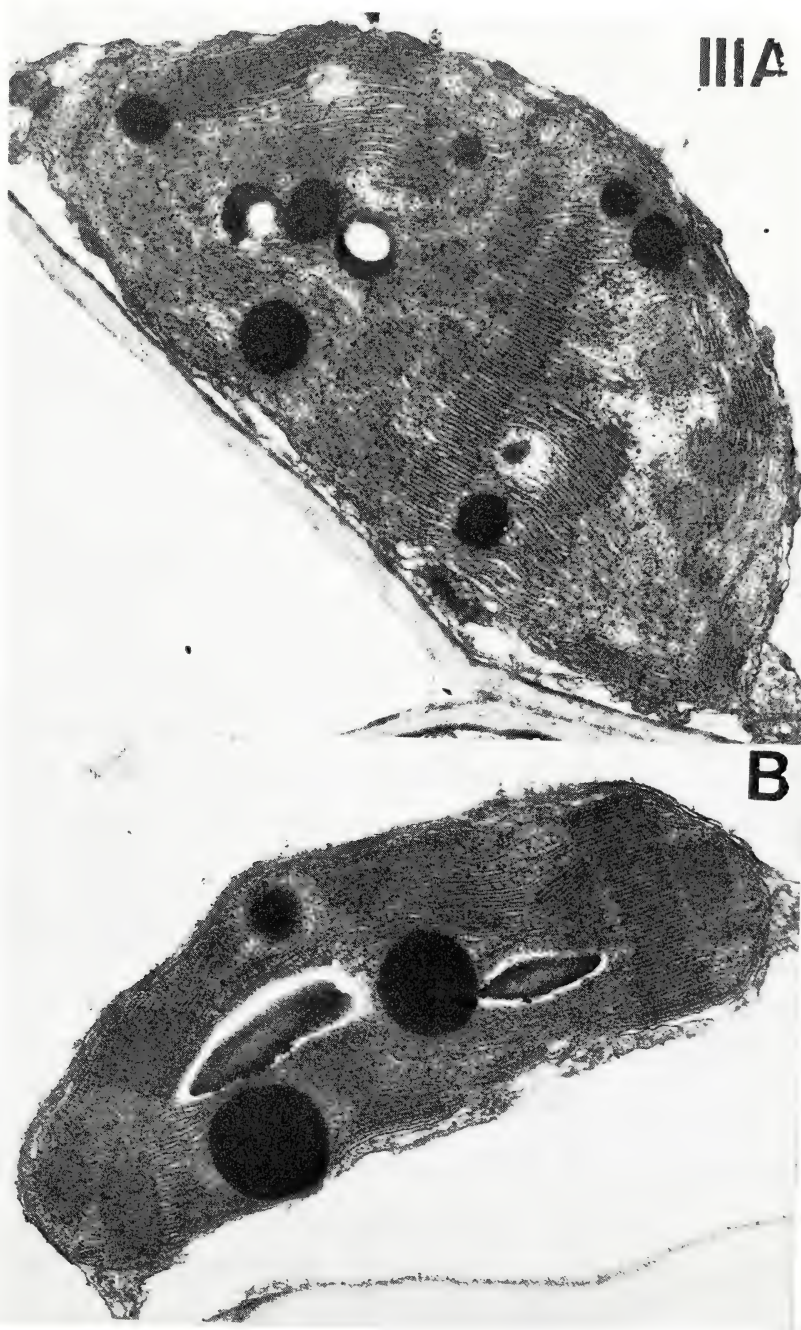


FIGURE 3. A. Sulfur-deficient chloroplast at the age of 8 weeks. Fixation and staining as for Fig. 2. X 25,000. B. Nitrogen-deficient chloroplast at the age of 8 weeks. Fixation and staining as above. X 35,000

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Aryl Sulphatases: Properties and Subcellular Distribution in Rat Liver¹

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Abstract

Enzymes of the arylsulphatase complex (Arylsulphatases A, B and C) were shown to be widely distributed throughout the endomembrane system of rat liver. Arylsulphatase C, a microsomal enzyme, was localized primarily in rough endoplasmic reticulum. Arylsulphatases A and B were more widely distributed. Electron microscope localization combined with cell fractionation studies showed the activity to be most concentrated in focal specializations of the bulk smooth endoplasmic reticulum or in lysosomes. Less concentrated enzyme activity was associated with rough endoplasmic reticulum, bulk smooth endoplasmic reticulum, the nuclear envelope and the Golgi apparatus (especially in secretory vesicles of the Golgi apparatus). Arylsulphatase A-B activity was not detected in mitochondria, microbodies, the plasma membrane and the ground cytoplasm and was low or absent from smooth endoplasmic reticulum of the Golgi apparatus zone. The results indicate a difference between Golgi apparatus-associated and the bulk of the smooth endoplasmic reticulum and raise the possibility of direct transfer of arylsulphatase A-B from rough endoplasmic reticulum to forming secretory vesicles of the Golgi apparatus.

The enzymes of the arylsulphatase group (aryl-sulphate sulphohydrolases, E.C. 3.1.6.1) are widely distributed among animals (1, 3, 4, 7, 8, 10-13, 15, 17, 28, 29, 31, 32, 34), microbial (16) and plant (29) tissues. The importance of sulphuric acid esters in many metabolic processes and disorders in animals (2-5, 8, 9, 17, 27, 28) adds considerable significance to the widespread distribution of the arylsulphatases. At least in animal tissues, the activity is largely confined to substrates in which sulphuric acid is conjugated with hydroxyl groups of phenols (Fig. 1). The inorganic sulfate released during the reaction can be precipitated with lead to form an electron dense reaction product. The latter provides a means for localizing the enzyme activity at the electron microscope level (1, 15, 18, 29, 34).

At least three arylsulphatases have been distinguished on the basis of their kinetic properties. In rat liver, two of the enzymes, Arylsulphatase A and Arylsulphatase B are considered to be localized primarily in the lysosome fraction (7, 8, 34). The third, Arylsulphatase C, is found in the microsome fraction (10, 13, 18). Yet, the precise subcellular distribution of these enzymes in liver has never been established. The purpose of this study was to investigate the subcellular distribution of Arylsulphatases A, B and C in rat liver with particular emphasis on components of the endomembrane system (25).

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Materials and Methods

Male rats, 200 to 250 g, 50 days old of the Holtzman strain, provided with laboratory chow and drinking water *ad libitum*, were sacrificed and the livers drained of blood. The livers were removed, minced at room temperature and used either for determination of arylsulphatase activity, cell fractionation studies or enzyme cytochemistry.

Golgi apparatus (20) and endoplasmic reticulum (6) were prepared as described previously. The heavy particulate fraction of Table 1 was obtained by collection of the band at the 1.5/1.6 M interface on a discontinuous sucrose gradient used for preparation of Golgi apparatus fractions (22). The band was removed, resuspended in homogenization medium and pelleted at 3,000 *g* for 30 min. This fraction is largely a mixture of mitochondria and rough-surfaced endoplasmic reticulum.

TABLE 1. *Arylsulphatase activities of isolated cell components of rat liver.*

Fraction	Enzyme Activity	
	Arylsulphatase C	Arylsulphatases A and B
	(mumoles <i>p</i> -hydroxyacetophenone/ mg protein/hr)	(mumoles <i>p</i> -nitrocatechol/ mg protein/hr)
Total Homogenate	59 ± 6	90 ± 13
Rough Endoplasmic Reticulum	385 ± 88	668 ± 58
Golgi Apparatus	87 ± 6	604 ± 13
Heavy Particulate	— —	574 ± 13

The substrate for Arylsulphatase A-B was *p*-nitrocatechol sulphate (Fig. 1) purchased from Sigma Chemical Company, St. Louis, Missouri. The reaction was carried out as described by Roy (31, 32) using a substrate concentration of 30 mM and a pH of 6.0 with a reaction time of 30 min unless indicated otherwise.

For determination of Arylsulphatase C activity, the substrate was potassium *p*-acetylphenylsulphate synthesized from *p*-hydroxyacetophenone and chlorosulfonic acid by an adaptation of the simplified method of Figenbaum and Neubert (14) for preparation of aromatic sulphuric acid esters. This substrate was chosen in preference to some readily available substances due to its relative stability toward metabolism by rat liver homogenates (11). Arylsulphatase C was assayed as described by Dogson *et al.* (12) with the exception that reactions were stopped after 30 min rather than after 1 hour. Proteins were determined by the biuret method.

Tissues and cell fractions subjected to cytochemical assay for arylsulphatases A-B were processed according to the method of Goldfischer (15), except that they were fixed overnight in 2% glutaraldehyde instead of being perfused as with the whole tissue experiments of Goldfischer (15). The tissues and fractions were then embedded in Epon

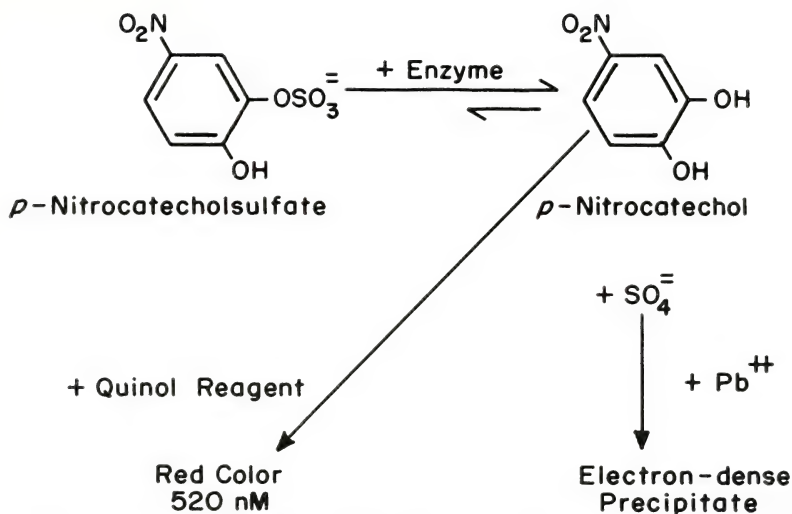


FIGURE 1. Summary of the detection methods employed for Arylsulphatase A-B. In the presence of the enzymes, the substrate p-nitrocatecholsulphate is cleaved to p-nitrocatechol plus sulphate. In the presence of quinol reagent, the p-nitrocatechol produces a red color absorbing at 520 nM. For cytochemical detection, the sulphate was complexed with lead to yield an electron-dense precipitate observable in the electron microscope.

(33) and thin sections were examined and photographed with a Philips EM 200.

Results

Whole homogenates of rat liver were used in establishing assay conditions for arylsulphatase A-B in rat liver. The activity was extremely stable and extracts could be stored for several months without loss of enzyme activity. Controls containing the enzyme solution with the substrate added immediately after incubation followed immediately by ethanol, and controls in which the substrate was incubated with buffer alone, were run simultaneously with experimental determinations.

Effect of time and enzyme concentration

Figure 2 shows the effect of incubating rat liver homogenates with the substrate in the presence of varying quantities of protein (Fig. 2A) and for varying times (Fig. 2B) under conditions of optimum pH (Fig. 2C) and a substrate concentration of 30mM. This substrate concentration was near optimal for Arylsulphatase C (12), supraoptimal (inhibitory) for Arylsulphatase A (31) and yielded first order kinetics for Arylsulphatase B (31, Fig. 2D). There was an approximately linear relationship between protein concentration and the reaction velocity over the range 0 to 4 mg of protein in the assay (Fig. 2A) and in subsequent studies a protein concentration of 1 mg or less was employed. In agreement with a previous observation (31), there was a decrease in reaction velocity during the first 10 min of incubation

after which the degree of hydrolysis was proportional to the time of incubation (Fig. 2B).

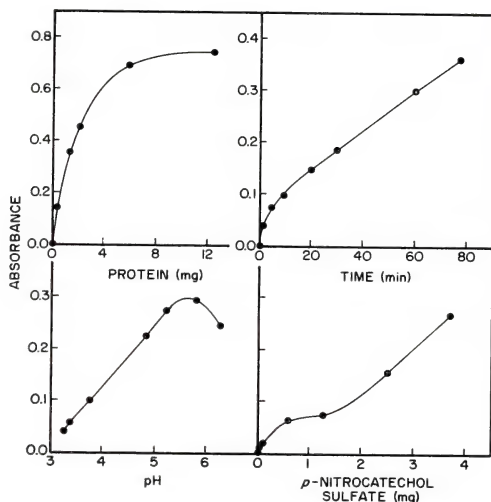


FIGURE 2. Properties of rat liver Arylsulphatase A-B assayed under standard conditions (31, 32) except for conditions varied showing increased absorbance due to release of p-nitrocatechol from p-nitrocatecholsulphate as a function of (A) protein concentration, (B) time, (C) pH, and (D) substrate concentration. The enzyme source was total rat liver homogenate (Table 1) frozen and thawed to release structure-linked latent enzyme activity.

Effect of pH

The effect of pH was determined over the range of 3 to 7. The pH optimum is that of Arylsulphatase B and was determined to be 5.7 in 0.15 M acetate buffer with nitrocatechol sulphate as substrate (31). The pH optimum for arylsulphatase A is 4.7 (31). The optimum for Arylsulphatase C is about pH 7.2 (10, 12)

Effect of substrate concentration

The effect of varying substrate on reaction velocity (Fig. 2D) shows a hump in the curve between 0 and 0.5 mg p-nitrocatechol sulphate which is due to the presence of Arylsulphatase A (12) which is saturated at a substrate concentration of approximately 3 mM. As shown previously (12), there is no obvious substrate optimum for arylsulphatase B within the limits studied (Fig. 2D).

Intracellular location

In tissue fractionation studies, the activities of Arylsulphatases A and B as compared to Arylsulphatase C showed distinctly different distribution patterns. Arylsulphatase C appeared to be specifically localized in the rough endoplasmic reticulum (Table 1). Arylsulphatases A-B, however, showed approximately equal distribution in the rough endoplasmic reticulum and the Golgi apparatus (Table 1).

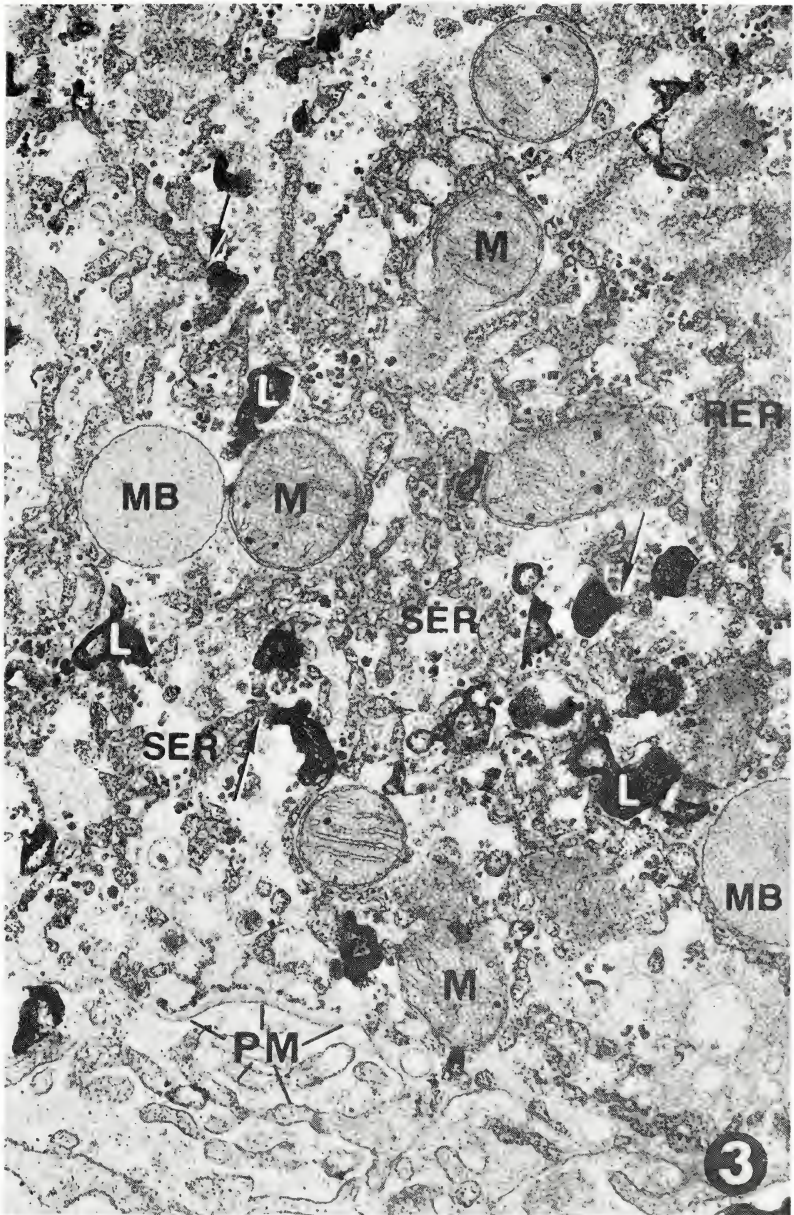


FIGURE 3. Electron micrograph of rat liver incubated for arylsulphatase activity at pH 5.5 for 30 min at 37°C (15). Thin sections were stained with lead citrate (30). Reaction product is found in specific bodies (L) adjacent to or continuous with (arrows) smooth endoplasmic reticulum (SER). Lighter, granular deposits of reaction product are seen over the lumens and membranes of both rough (RER) and smooth endoplasmic reticulum. The plasma membrane (PM) at the cell border, mitochondria (M), microbodies (MB) and the cytoplasmic matrix are free of reaction product. Magnifications are approximate. X 30,000.

This distribution was confirmed and extended by the results from cytochemical localization of Arylsulphatases A-B. The heaviest deposits of lead were found over the membranes and contents of bodies attached to and interspersed with fields of smooth endoplasmic reticulum (Fig. 3). These bodies may represent lysosomes but their consistent association with smooth endoplasmic reticulum was unexpected. More important in the context of the present study, lead deposits of a more diffuse nature were found equally distributed over the lumens of both rough and smooth endoplasmic reticulum (Figs. 3 and 4), the nuclear envelope (Fig. 4) and cisternae and vesicles of the Golgi apparatus (Fig. 4). In isolated fractions of rough endoplasmic reticulum, dense reaction products were still present and uniformly distributed but more so over the membranes than within cisternal lumens (Fig. 5). With isolated Golgi apparatus fractions, reaction products were found over secretory vesicles containing lipoprotein secretory products as well as secretory vesicles lacking lipoprotein secretory products and presumed to represent primary lysosomes (Figs. 6 and 7). Only small amounts of reaction product were found over Golgi apparatus cisternae and the smooth system of peripheral tubules (23, 24, 26). Both the isolated endoplasmic reticulum and Golgi apparatus fractions were free of the lysosome-like bodies containing dense lead deposits observed *in situ* (Fig. 3).

Discussion

In the animal tissues investigated by Goldfisher (15) and others (1, 34), Arylsulphatase A-B activity was largely localized in cytoplasmic bodies identified as lysosomes. In contrast, Arylsulphatase C has been generally considered to be a microsomal enzyme (10, 18), but microsomes are a heterogeneous cell fraction containing membrane fragments of many different types of cell components. The analysis of arylsulphatase activity is complicated to some extent by anomalous kinetic properties. These anomalies have been noted previously (31, 32) and no clarification is provided by the present study.

Our studies, however, do show that much of the Arylsulphatase A-B activity of rat liver is localized in cytoplasmic structures not readily identified as conventional lysosomes. Arylsulphatase A-B activity was about equal in endoplasmic reticulum and Golgi apparatus fractions. Both fractions were considerably enriched over that of the homogenate but the specific activity was only 0.1 that reported for lysosome fractions (7, 8). Its presence in the endoplasmic reticulum and Golgi apparatus fractions was not due to lysosomal contamination of the fractions as shown by the cytochemical analyses of the isolated fractions (Figs. 6 and 7).

In contrast, Arylsulphatase C activity of the Golgi apparatus fraction was only 22% that of the rough endoplasmic reticulum (Table 1). This value is higher than that observed with glucose-6-phosphatase and other endoplasmic reticulum enzymes (13 to 15%, ref. 21). One explanation for this discrepancy lies in the observation that potassium

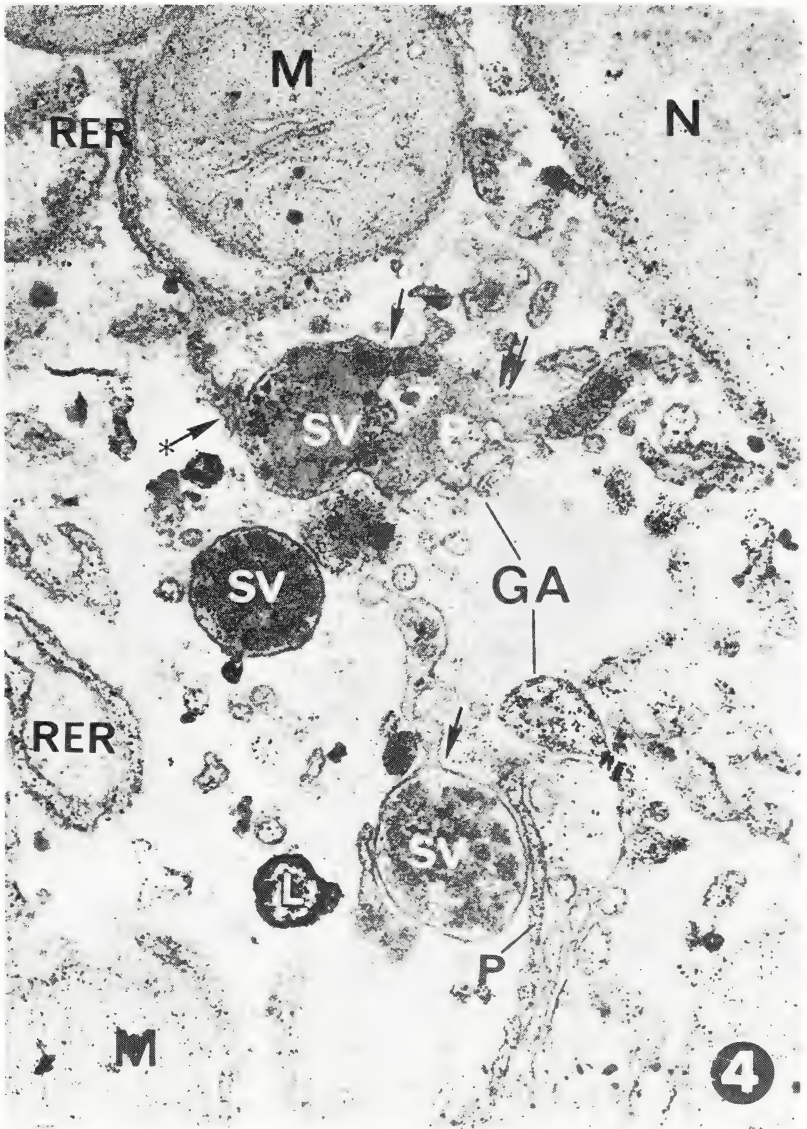


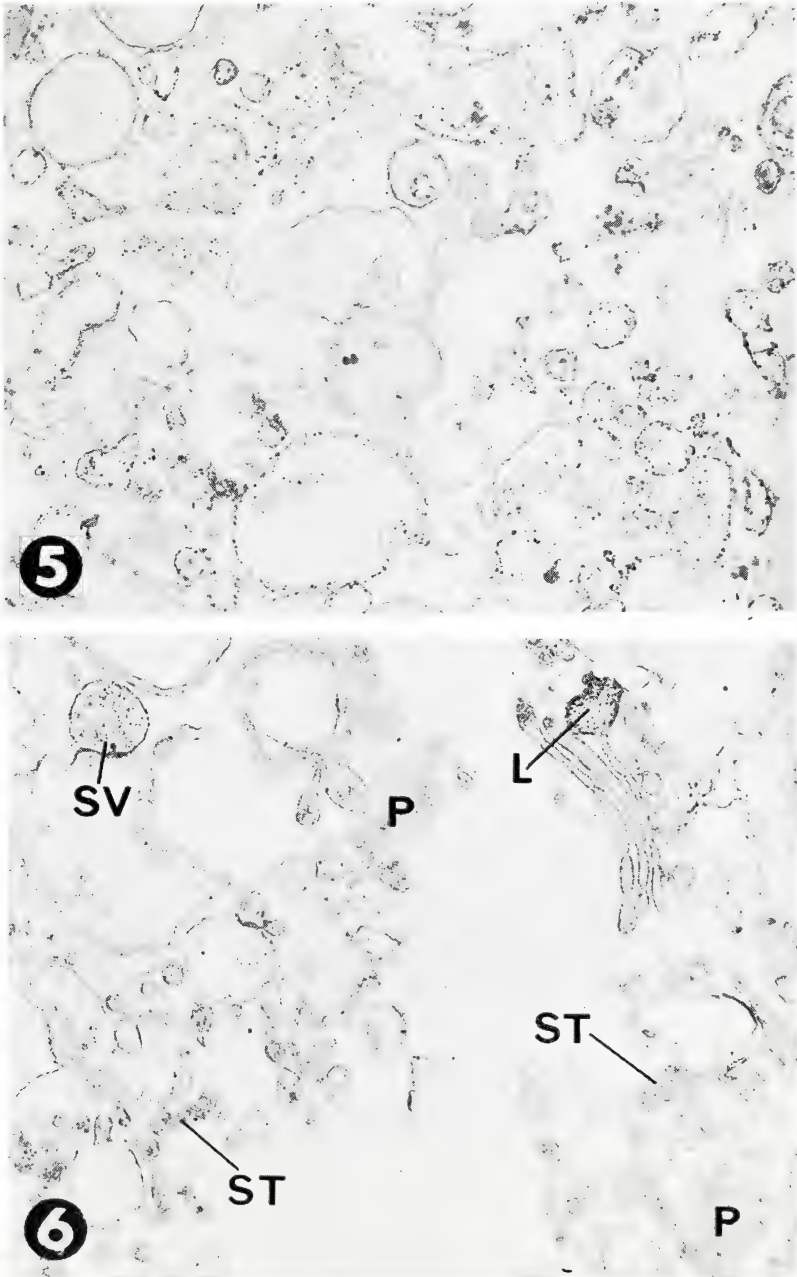
FIGURE 4. As in Figure 3 showing a portion of the Golgi apparatus (GA) adjacent to the nucleus (N). Granular reaction product is seen within the lumen of the nuclear envelope, within plate-like portions (P) of Golgi apparatus cisternae, within secretory vesicles (SV) of the Golgi apparatus and within the lumens of rough endoplasmic reticulum (RER). One densely staining body next to the Golgi apparatus may represent a primary lysosome (L). Note the connections between lipoprotein-containing, smooth endoplasmic reticulum tubules and the large secretory vesicles of the Golgi apparatus (single arrows), between these tubules and a portion of a Golgi apparatus plate (white P) seen in face view (double arrow) and between a secretory vesicle and rough endoplasmic reticulum (arrow with asterisk). Arylsulphatase reaction product is reduced somewhat in amount from the SER tubules near their point of continuity with Golgi apparatus cisternae. M mitochondrion. X 54,000.

p-acetylphenylsulphate is not a completely specific substrate for Arylsulphatase C under the conditions used, so that the arylsulphatase A and B, shown to be present in the Golgi apparatus, could account for the additional 7 to 10% of the activity. F. A. Rose (personal communication) suggested that phosphate ions increase the specificity of *p*-acetylphenylsulphate for Arylsulphatase C. However, phosphate ions were not added to our reaction mixture.

In the cytochemical analyses, the activity detected may be largely ascribed to Arylsulphatase B. Arylsulphatase A-B show a high degree of substrate specificity hydrolyzing *p*-nitrocatechol sulphate while splitting *p*-acetylphenylsulphate very slowly (31, 32). The pH optima of these two activities also differ. Type C Arylsulphatase has a pH optimum slightly over neutrality and would not be expected to contribute to the cytochemical results. At the acid pH employed, both Arylsulphatases A and B are active although data of Figure 2D suggest that, in future studies, Arylsulphatases A and B could be differentiated cytochemically by varying substrate concentration.

In addition to the focal specializations of smooth endoplasmic reticulum (Fig. 3) and other lysosome-like bodies, we find arylsulphatase activity distributed throughout the endomembrane system (Figs. 3 to 7). It is less evident in the smooth tubules of the Golgi apparatus (Figs. 4, 6 and 7) and is absent from plasma membrane, microbodies, mitochondria and ground cytoplasm. It is present in the nuclear envelope, bulk rough and smooth endoplasmic reticulum and cisternae and vesicles of the Golgi apparatus. This distribution is similar to that observed in plants where acid arylsulphatase activity was found in the nuclear envelope and endoplasmic reticulum with the lead deposits located in the lumen lying next to the membrane (29). Poux (29) noted lead deposits in a type of cytoplasmic body found in root tips of *Cucumis sativus* which closely resemble the structures illustrated in Figure 3 but which she tentatively identifies as phragmosomes (= microbodies, ref. 19). We found no arylsulphatase activity associated with microbodies in rat liver. Nor did we find arylsulphatases concentrated in pericanalicular dense bodies of liver in contrast to the report by Abraham (1). Arylsulphatase A-B was not found cytochemically in mitochondria. The activity present in crude mitochondrial fractions isolated from liver (31, 32, Table 1) may be due to contamination of the fraction by lysosomes and/or endoplasmic reticulum.

The widespread localization of Arylsulphatase A-B in the endomembrane system of rat liver raises the possibility of an active protein being synthesized in the endoplasmic reticulum and/or nuclear envelope and being transported to the bulk smooth endoplasmic reticulum or to the Golgi apparatus where it then becomes incorporated into lysosomes and other types of vesicles. Of specific interest in terms of the present study



FIGURES 5 and 6. Isolated cell fractions from rat liver incubated for Arylsulphatase A-B activity as described in Figure 3. The only lysosome-like bodies observed in the preparations were those attached to Golgi apparatus cisternae. FIGURE 5. Endoplasmic reticulum fraction showing finely granular reaction product located principally over the membranes or within the cisternal lumens against the membranes. X 40,000. FIGURE 6. Golgi apparatus fraction, showing finely granular reaction product primarily restricted to secretory vesicles containing lipoprotein particles (SV) and vesicles lacking the particles and presumed to represent primary lysosomes (L) still attached to the Golgi apparatus. In this preparation, both the plate-like portions of cisternae (P) and the lipoprotein-containing SER tubules (ST) lacked reaction product. X 33,000.

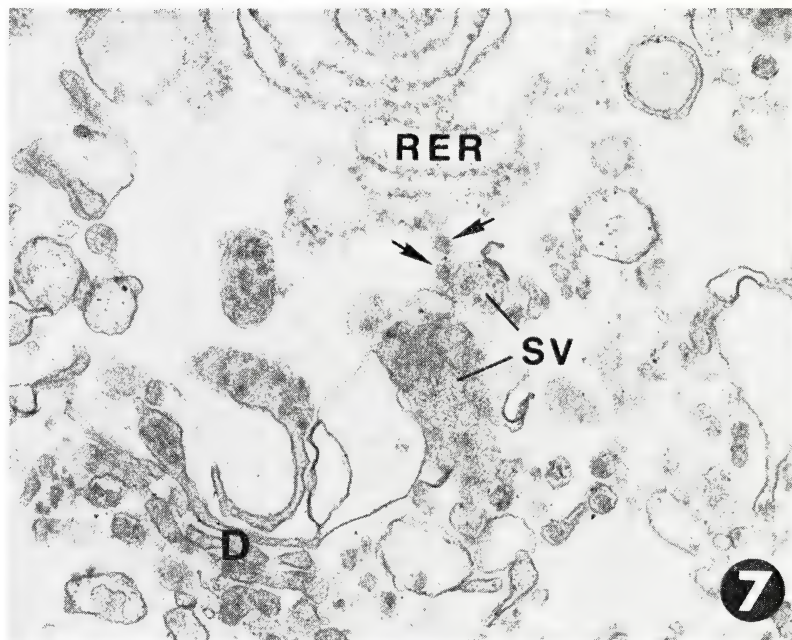


FIGURE 7. As in Figure 6 showing an intact stack of Golgi apparatus cisternae = dictyosome (D) with finely granular reaction product being restricted to the secretory vesicles (SV) and a fragment of rough endoplasmic reticulum (RER). A region of close association between the RER fragment and a portion of a secretory vesicle is indicated by the arrows. Rather than being represented by a continuous tubule, the region of association has a beaded or vesicular character. X 47,000.

is the absence of arylsulphatase activity from smooth tubules (= smooth endoplasmic reticulum, refs. 23, 24, 26) of the Golgi apparatus zone. The results show a difference between Golgi apparatus-associated and bulk smooth endoplasmic reticulum and raise the possibility of direct transfer of arylsulphatase A-B from rough endoplasmic reticulum to forming secretory vesicles of the Golgi apparatus.

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**Constancy of Unsaturation in Molecular Species of
Cardiolipin and Phosphatidyl Ethanolamine
from Beef Heart Mitochondria¹**

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Abstract

The diglyceride acetate products of the acetolysis of beef heart mitochondrial cardiolipin were separated into three fractions, trienes, tetraenes and pentaenes, by argentation chromatography. Tetraenes accounted for 64 per cent of the total diglyceride acetates and linoleic acid accounted for 93 per cent of the total acyl residues of this fraction. There was an inverse linear relationship between the oleic and linoleic acid contents of cardiolipin preparations. Six diglyceride acetate fractions were obtained from beef heart mitochondrial phosphatidyl ethanolamine. The major fraction, tetraenes, accounted for 54 per cent of the total diglyceride acetates. Stearic and arachidonic acids accounted for 89 per cent of the acyl groupings in this fraction. There was a high degree of consistency in the total unsaturated fatty acid content of cardiolipin preparations and in all diglyceride acetate fractions derived from cardiolipin. This consistency was also evident in phosphatidyl ethanolamine preparations.

In a preliminary communication we reported that the fatty acids were nearly randomly distributed between the α - and β -positions of beef heart cardiolipin (8). Argentation chromatography of the diglyceride acetates prepared by acetolysis of cardiolipin revealed the presence of three molecular species. The major species, tetraenes, contained more than 90% linoleic acid (8). Wood and Harlow (19), by gas chromatographic separation, similarly found one predominant diglyceride in the Phospholipase C digestion products of rat liver cardiolipin. In continuing our earlier studies, analyses of multiple cardiolipin preparations revealed a great deal of constancy in molecular species distribution. Further, this constancy was also observed in the total unsaturated fatty acid content of both native cardiolipin and the molecular species derived therefrom. These results are reported herein along with those obtained from parallel analyses of beef heart mitochondrial phosphatidyl ethanolamine (PE). Next to cardiolipin, PE contains a higher proportion of unsaturated acyl residues than do other beef heart mitochondrial phospholipids and contains high levels of arachidonic acid (1,2,6). Analysis of PE was thus of particular interest in view of the hypothesis of De Pury and Collins (4) that arachidonyl species are more firmly bound to mitochondrial structural protein than are other species. This hypothesis is correlated in part with

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our recent observation that the highly unsaturated cardiolipin is tightly bound to cytochrome oxidase and is essential for function of the enzyme (1, 2).

Materials and Methods

Beef heart mitochondria were isolated by the method of Löw and Vallin (10). Cardiolipin and PE were isolated from washed total lipid extracts (1) by preparative thin-layer chromatography (8). Analysis by two-dimensional thin-layer chromatography (12) revealed these lipids to be free of other polar lipid contaminants. To minimize autotoxidation, samples were stored in a nitrogen atmosphere at -20° C and a small amount of butylated hydroxyanisole was added to all solvents used. Diglyceride acetates were prepared by acetolysis at 145° C for 40 hours in a nitrogen atmosphere according to Renkonen (14). In certain cases, PE was mixed with sphingomyelin (chromatographically pure, Sigma Chemical Company), digested with Phospholipase C from *Clostridium welchii*, and the diglycerides thus obtained were acetylated (14). The diglyceride acetates were first purified by thin-layer chromatography (8) and then separated by argentation chromatography on thin layers of Silica gel G containing silver nitrate (10:3 by weight). Plates were developed in chloroform containing from 0.75 to 1.25% methanol (9, 13). Lipid bands were localized, recovered (8), methylated and analyzed by gas chromatography (7). Relative concentrations of various diglyceride acetates was determined by ester analysis of the material eluted from silver nitrate plates (18).

Acetolysis was chosen for the preparation of diglyceride acetates as a matter of convenience. It is known that intramolecular migration of fatty acids occurs under these conditions (11, 15). However, intermolecular migration does not occur during this reaction (14) and, since positional distribution of fatty acids was not determined, the diglyceride acetates were suitable for analysis of molecular species distribution. As a check on the method, diglyceride acetates prepared by acetylation of the products of Phospholipase C digestion of PE (in the presence of sphingomyelin) gave results nearly identical to those obtained with the acetolysis product of the same PE sample in all analyses reported herein. Cardiolipin was not digested by the enzyme under these conditions. Although there was oxidative degradation on acetolysis, as evidenced by darkening of the reaction mixture, the colorless diglyceride acetates isolated had a fatty acid composition identical to that of the starting lipid.

Results and Discussion

Only three fractions were obtained on separation of the cardiolipin diglyceride acetates. The predominant molecular species in each fraction were trienes, tetraenes, and pentaenes, respectively (Table 1). A remarkably constant amount of each species was present in the seven mitochondrial preparations analyzed. Tetraenes were the major species encountered, representing 64% of the total (range 60 to 67%). In contrast, a total of six fractions were obtained on argentation chromato-

graphy of PE (Table 1). Saturates (minor) *plus* monoenes *plus* dienes together accounted for approximately 28% of the total. As with cardiolipin, tetraenes were the major molecular species, accounting for 54% of the total. There was a reasonable consistency in distribution of molecular species among the four separate preparations analyzed.

TABLE 1. *Distribution of molecular species of diglyceride acetates derived from cardiolipin and phosphatidyl ethanolamine of beef heart mitochondria.*

Molecular species ²	% of Total ¹	
	Cardiolipin (7) ³	Phosphatidyl ethanolamine (4)
Saturates—dienes	—	27.6 ± 4.25
Trienes	20.5 ± 1.86	6.9 ± 1.72
Tetraenes	63.8 ± 2.66	54.4 ± 2.97
Polyenes	15.7 ± 1.71	11.1 ± 2.28

¹Results, expressed as % of total ester groups recovered, are reported as the average ± standard deviation.

²Fractions are designated as the total number of double bonds.

³Number of separate mitochondrial preparations analyzed.

In all cardiolipin preparations, at least 91% of the total fatty acyl groupings were unsaturated (average 95%, range 91 to 99%) (Table 2). There was, however, appreciable variation in individual fatty acids comparing different samples, particularly with regard to the major fatty acid, linoleate. There was also appreciable variation among the individual fatty acids of each diglyceride acetate species from cardiolipin comparing separate preparations (Table 2). As with the parent lipid, there was a reasonable consistency in the total percentage of unsaturated fatty acids, which on the average accounted for over 90% of the total acyl residues in all molecular species. Linoleic acid was the major fatty acid in all fractions. In the tetraene fraction linoleate accounted for, on the average, 93% of the total fatty acids.

Table 2 contains data on the fatty acid composition of PE and the more highly unsaturated diglyceride acetate fractions derived therefrom. As with cardiolipin, although there was variation in individual fatty acids, the total unsaturated acid content was nearly constant comparing different preparations and averaged 64% (range 61 to 68%). The major acids were stearate and arachidonate. This constancy in unsaturated acid content was also evident in the major subfractions analyzed. In the major species, tetraenes, stearate and arachidonate together accounted for approximately 89% of the total acyl residues. We had previously demonstrated that arachidonate is contained nearly entirely in the β -position of beef heart mitochondrial PE (8). Thus it can be concluded that 1-stearoyl-2-arachidonyl phosphatidyl ethanolamine is a major species of beef heart mitochondria.

The diphosphatidyl glycerol structure of cardiolipin precludes conclusions on the molecular species distribution of the parent

phosphatide based on results obtained with diglyceride acetates. However, the results contained herein suggest that the majority of the molecular species contain three linoleate molecules and further imply the occurrence of a molecular species containing solely linoleic acid.

TABLE 2. *Fatty acid composition of cardiolipin, phosphatidyl ethanolamine, and their diglyceride acetates*¹.

Cardiolipin (7) ²				
Acid	Total	Trienes	Tetraenes	Polyenes
14:0	0.3 ± 0.2	2.1 ± 2.23	0.4 ± 0.6	0.8 ± 0.66
16:0	2.3 ± 1.40	5.4 ± 1.68	1.8 ± 0.95	5.1 ± 1.86
16:1	2.5 ± 0.17	11.4 ± 1.15	1.0 ± 0.68	4.0 ± 1.16
18:0	2.1 ± 1.47	2.2 ± 1.23	0.9 ± 0.51	3.3 ± 1.37
18:1	9.9 ± 3.14	28.5 ± 1.77	1.5 ± 0.94	22.0 ± 7.05
18:2	73.9 ± 5.72	50.0 ± 6.58	93.2 ± 4.32	37.2 ± 3.86
18:3	5.8 ± 1.42	0.4 ± 0.63	1.1 ± 1.85	20.4 ± 6.61
20:3	2.0 ± 1.41			3.8 ± 1.39
20:4	1.5 ± 0.97			3.3 ± 1.38
20:5	Trace			0.2 ± 0.46
22:5				
22:6				
Total % unsaturated	95.3 ± 2.93	90.4 ± 6.78	96.7 ± 1.72	90.9 ± 3.56

Phosphatidyl Ethanolamine (5)				
Acid	Total	Trienes	Tetraenes	Polyenes
14:0	0.9 ± 1.34	1.3 ± 1.48	0.2 ± 0.24	0.2 ± 0.87
16:0	3.5 ± 2.03	16.4 ± 9.56	1.9 ± 0.65	5.9 ± 2.48
16:1	1.0 ± 0.86	7.0 ± 2.40	0.6 ± 0.47	1.8 ± 1.41
18:0	32.5 ± 5.81	15.1 ± 6.36	40.3 ± 2.48	33.5 ± 6.67
18:1	4.1 ± 0.89	24.6 ± 4.18	1.8 ± 2.14	10.6 ± 2.58
18:2	11.5 ± 4.32	31.9 ± 9.45	4.7 ± 4.38	4.3 ± 1.11
18:3	2.5 ± 3.29	0.6 ± 1.25	0.5 ± 0.58	0.8 ± 1.07
20:3	2.5 ± 1.55	3.1 ± 3.78	1.7 ± 0.40	0.1 ± 0.31
20:4	35.6 ± 2.31		48.5 ± 5.60	16.1 ± 7.14
20:5	4.8 ± 0.42			18.8 ± 8.04
22:5	0.9 ± 0.80			4.2 ± 5.70
22:6	0.5 ± 1.07			3.8 ± 3.71
Total % unsaturated	63.8 ± 3.40	67.2 ± 5.06	57.8 ± 2.48	60.5 ± 8.63

¹Weight % of fatty acids reported as the average ± standard deviation.

²Number of separate mitochondrial preparations analyzed.

The observations on the constancy in total percentage of unsaturated acyl residues in both PE and cardiolipin and the molecular species derived from them render feasible the conclusion that the physiological function of these lipids requires a closely controlled content of unsaturated fatty acids. This conclusion is substantiated by the observation that alterations in fatty acid composition can influence the properties of membranes by affecting both the attractive forces between lipids and proteins and the molecular packing of membrane lipids (4, 16).

Eichberg (5) has recently reported that nearly 50% of the cardiolipin of beef heart can be isolated as a protein complex. The high degree of unsaturation in all diglyceride acetate species from cardiolipin may explain the tenacious binding of cardiolipin to protein as being due to extensive interaction between the double bonds of the fatty acids and hydrophobic groups within the proteins. (3, 4, 17).

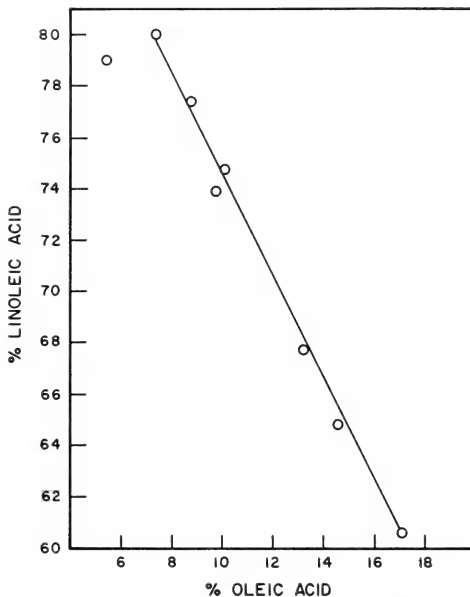


FIGURE 1. Relationship between the contents of linoleic acid and oleic acid in beef heart mitochondrial cardiolipin. Data are weight percentages calculated by peak area analysis of gas chromatograms.

We wish to call attention to one further observation made in the course of these studies. Although there was appreciable variation in the contents of oleic and linoleic acids comparing different preparations of cardiolipin, there was a nearly linear inverse relationship between the contents of these two acids (Fig. 1). This appears to indicate that at least a portion of one acid may be replaced by the other in cardiolipin without impairment of the psychochemical properties of the mitochondrial membrane.

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CHEMISTRY

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RICHARD PILGER, Department of Chemistry, St. Mary's College,
Notre Dame, Indiana 46556, was elected Chairman for 1972

ABSTRACTS

The Synthesis and Decomposition of Some Interesting 1-Pyrazolines. LEO MULLINS, TERRY L. KRUGER, THERESA CHENG, and JOHN SHARP, Department of Chemistry, Ball State University, Muncie, Indiana 47306. —The synthesis of several bicyclo-1-pyrazolines through 3+2 cycloaddition has been studied. The kinetics of the decomposition of a few of these compounds has been carried out with the identification of the decomposition products. These studies have shown that the decompositions may occur by processes allowed by orbital symmetry considerations. Future work includes product analysis, kinetics, mass spectra, and comparison of photodecomposition products with thermal-decomposition products.

Properties and Reactions of Some N, N-Diethylaniline Oxides. TERRY L. KRUGER, NANCY E. THARP and JAMES W. KRESS, Department of Chemistry, Ball State University, Muncie, Indiana 47306. —The title compounds with a variety of ring substituents have been synthesized. The basicities of these water soluble compounds have been correlated in the Hammett Equation. These compounds undergo facile Cope elimination to the corresponding N-ethylphenylhydroxylamines. An analysis of some aspects of the mechanism of this reaction and an explanation of the effect of substituent on both this reaction and basicity was presented.

Synthesis and Identification of p-Benzoquinones Occurring in Arthropod Defensive Secretions. JOSEPH E. ROGERS, JR., BENJAMIN FIESELMANN and JAMES JOSE, Department of Chemistry, Earlham College, Richmond, Indiana 47374. —Eight substituted p-benzoquinones known to occur in arthropod defensive secretions have been prepared. Chromatographic methods have been explored which facilitate identification of these quinones in complex naturally-occurring secretions. The eight quinones (p-benzoquinone, methyl-p-benzoquinone, ethyl-p-benzoquinone, 2,3-dimethyl-p-benzoquinone, 2,5-dimethyl-p-benzoquinone, 2,3,5-trimethyl-p-benzoquinone, 2-methoxy-p-benzoquinone, and 2-methyl-3-methoxy-p-benzoquinone) were either purchased or in most cases were synthesized by oxidation of suitably substituted amines or phenols. The gas chromatographic behavior of these quinones has been investigated on several stationary phases over a range of temperatures. All but two of the quinones may be resolved on a five-foot by one-eighth inch copper column containing 2 per cent OV-17 on GC Q. Thin-layer and paper chromatography of the 2,4-dinitrophenylhydrazones derivatives of the quinones reveals that all eight 2,4DNP derivatives may be resolved on a silica gel layer (Eastman Chromagram K301R) upon development with a 3:1 chloroform:xylene solvent in the presence of aqueous ammonia vapors.

A Chemical Study of the White River at Muncie. CHARLES T. BOTKIN and ROBERT E. VAN ATTA, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—Analyses for several chemical components of the White River are reported for a 50-day period during the summer of 1971. The objective of the study was to determine whether or not significant variations in dissolved oxygen, chloride, free and albuminoid ammonia, pH, calcium, magnesium, iron, and several other factors occurred and could be conveniently monitored in a flowing system of considerable volume. Results are presented for sampling points upstream and downstream of the city and are compared with climatological records for the sampling period. Time versus analyzed factor profiles are presented and, where feasible, correlated with each other and with other variables in the stream's composition.

A New Experiment for Advanced Inorganic Chemistry. B. N. STORHOFF and D. F. STORHOFF, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—The utility of simple group-theoretical considerations in inorganic chemistry is well documented. The appreciation for such considerations has prompted the design of an experiment which illustrates their utility in vibrational spectroscopy. The experiment focuses on the infrared active CO stretching vibrations of several manganese carbonyl complexes. Probable structures are assigned to the complexes by comparing the observed and predicted spectra.

Rhenium(I) Complexes of 2-Cyanoethyldiphenylphosphine. B. N. STORHOFF, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—Halopentacarbonylrhenium (I) (Cl or Br) reacts with 2-cyanoethyldiphenylphosphine(L) to yield novel complexes of the stoichiometry $[\text{Re}(\text{CO})_5\text{LX}]_n$. Infrared data and molecular weight data suggest that these complexes are dimeric and utilize L as a bridging ligand. The complexes react with a variety of donor solvents and ligands (L') to yield complexes of stoichiometry $\text{Re}(\text{CO})_5\text{LL}'\text{X}$.

Chelating Behavior of Some New Sterically Hindered Tetrafunctional Azine Ligands. JOHN A. SCARLETT and WILMER J. STRATTON, Department of Chemistry, Earlham College, Richmond, Indiana 47374.—Two new unsymmetric azine ligands and their iron(II) complexes have been synthesized and characterized. Pyridinalpyridylphenylketazine was found to form two complexes as expected: $[\text{Fe}_2\text{L}_3]\text{I}_4$, with each ligand bridging two metals in a bis-bidentate fashion, and $[\text{FeL}_2]\text{I}_2$, with tridentate bonding of each ligand. Elemental analysis, infrared spectra, and electronic spectra were used to confirm these structures. Pyridylmethylpyridylphenylketazine also forms a bridged complex, $[\text{Fe}_2\text{L}_3]\text{I}_4$, but apparently does not form a 2:1 tridentate complex. Attempts to produce the latter complex resulted only in formation of a hydrazone complex. A ligand hydrolysis mechanism is proposed, in which the steric effects of the phenyl and methyl groups cause the ligands to "squeeze" out a metal ion, thus allowing proton attack on the uncoordinated C=N group.

Conductivity Studies in Acetonitrile for a Novel Series of Metal Chelates with 2-Pyridylmethylketazine. DAVID J. HARRIS and WILMER J. STRATTON, Department of Chemistry, Earlham College, Richmond, Indiana

47374.—Complexes of NiCl_2 , CoCl_2 , ZnCl_2 with 2-pyridylmethylketazine, each of which had analyses indicating 4/5 or 5/6 of a ligand per metal, were studied in acetonitrile solution. The data obtained showed that the conductances of the three compounds are distinctly different. Empirical equivalent weights were established from the conductivity data for each complex. Comparison of the slopes of plots of equivalent conductance versus square root of concentration with similar plots for two reference compounds was used to establish the charge types of the complexes. Results for the nickel complex are consistent with a formula $[\text{Ni}_2\text{L}_2\text{Cl}_3(\text{H}_2\text{O})_2][\text{NiCl}_4]$. Data for the cobalt and zinc complexes do not fit this formulation and are indicative of a more highly polymeric structure.

A Counterdiffusion Study of Ferric and Silicate Ions in Agar Media.

JON D. NAYLOR and JOHN H. MEISER, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—The development of new techniques for obtaining quantitative data used in the investigation of the counterdiffusion of ferric and silicate ions in an agar gelatin is presented. A treatment is given of the equations used for predicting the location of the band precipitates at given time intervals based on the supersaturation theory. The authors also presented a brief historical account of the Liesegang Phenomenon and a review of several theories providing explanations of this occurrence of band precipitates.

Liquid X-Ray Diffraction Studies of the Structures of Aqueous Metal Nitrate, Acetate, and Perchlorate Solutions Using Overviewing Techniques.

RICHARD M. LAWRENCE and JAN R. RELFORD, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—Overviewing techniques were developed to examine the structures of liquids by x-ray diffraction. Viewing the entire scattering volume of a sample yields agreement between the forms of the observed and calculated scattering curves and permits routine calculation of a radial distribution function for the system. In these studies radial distribution functions were calculated for almost twenty aqueous solutions of silver, lead, thallium (III), mercury (I), and cerium (III) nitrates, acetates, and perchlorates. These radial distribution functions are interpreted in terms of the numbers and distances of metal-metal and metal-oxygen interactions among closest neighbors. The mercury (I) solutions are the only solutions studied in which there are significant metal-metal contacts, and the results are consistent with the presence of Hg_2^{2+} . In the other solutions studied the metal ions appear to be in contact with from four to nine oxygens of water molecules or oxy anions. The number of metal-oxygen contacts is found to vary in a consistent manner with the concentration of the solution and the nature of the cation and the anion. The metal-oxygen distances agree with the sums of the van der Waals radius of oxygen and the crystal ionic radii of the metal ions.

Molecular Orbital Calculations on Some Nitrogen Heterocycles.

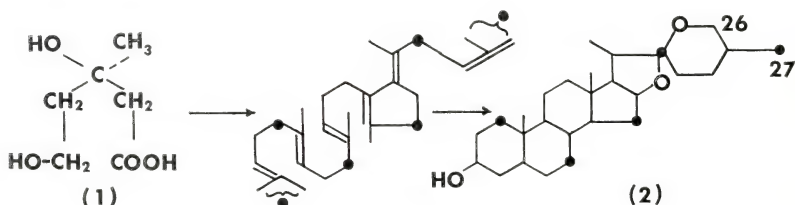
T. L. KRUGER and JAMES W. KRESS, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—A CNDO/2-INDO calculation of the quantum-mechanical properties of diazomethane and some substituted pyrazoles and 1-pyrazolines was presented. Calculated and experimental

bond orders, bond lengths, ultraviolet spectra and their relationship to the physical properties of the compounds were compared. Specific emphasis was placed on comparison of HMO and CNDO/2-INDO prediction for pyrazole and substituted pyrazoles.

An Anticholinesterase Agent and Myasthenia Gravis. ROBERT M. BROOKER, Department of Chemistry, Indiana Central College, Indianapolis, Indiana 46227, and T. LAMAR KERLEY, Dow Human Health R and D Laboratories.—Large doses of impure para-xylene-bis (pyridinium) chloride when given to unanesthetized dogs produced a muscle weakness that progressed to complete paralysis. Small doses of the drug reversed the paralytic effects of d-tubocurarine. It was found that this latter effect was caused by an impurity present in the original salt. Attempts were made to isolate and identify this compound that exhibited this anticholinesterase activity.

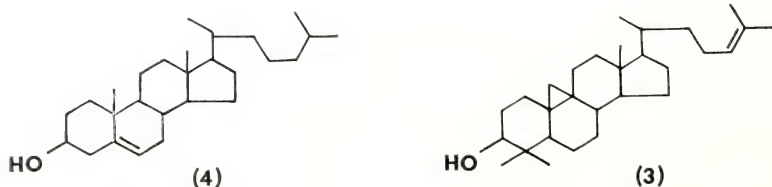
The Biosynthesis of Steroidal Sapogenins. PAUL ANDERMATT, University of Basel, Switzerland, and Department of Chemistry, Earlham College, Richmond, Indiana 47374.—The biosynthesis of steroids in human and animal organisms is known: acetic acid $\rightarrow$ mevalonic acid $\rightarrow$ squalene $\rightarrow$ cholesterol, or a similar C_{27} -steroid.

From *Digitalis lanata*, an European plant, tigogenin (2) was isolated. After the incorporation of (2- ^{14}C)-mevalonic acid (1) the molecule showed the expected distribution of radioactivity:



The distribution of radioactivity was determined by chemical degradation of the molecule.

The cyclisation and ring closure are strictly stereospecific. One fifth of the radioactivity was located at C-27, while C-26 showed no radioactivity. To explain this stereospecific ring closure, a Δ^{24} -steroid as desmosterol or cycloartenol (3) may be a possible precursor of tigogenin. To clarify this problem, radioactive cycloartenol (3) and cholesterol (4) were fed to the plants. Whereas cholesterol was incorporated, tigogenin showed no radioactivity after the application of cycloartenol.



These results show, that the biosynthesis of tigogenin involves the following steps: mevalonic acid $\rightarrow$ squalene $\rightarrow$ cholesterol $\rightarrow$ tigogenin.

Thin-Layer Gel Filtration Studies of Adenosine Deaminases from Beef and Beef Fetus Livers¹. BRUCE A THORNBURGH and PANG FAI MA, Department of Chemistry, Ball State University, Muncie, Indiana 47306. —The chromatographic patterns of adenosine deaminase in beef liver and beef fetus liver showed that the enzyme exists in two forms in these tissues. The high molecular weight enzyme (Type A) was the predominant form in the beef liver and the low molecular weight enzyme (Type C) was the predominant form in the beef fetus liver. The Type-A enzyme was found to partially dissociate to the Type-C enzyme when it was rechromatographed on a Sephadex column.

A rapid method for detecting the presence of the Type-A and Type-C enzymes was needed to study the dissociation of the Type-A enzyme under various conditions. This led to the development of a thin layer gel filtration chromatographic technique which was applicable to adenosine deaminase. The technique involved the detection of the ammonia that is given off in the deamination reaction with Nessler's Reagent.

¹This study was supported by a Student-Faculty Research Grant from Ball State University.

A Comparative Study of Adenosine Deaminase in Human Tissues.¹ THOMAS A. MAGERS and PANG FAI MA, Department of Chemistry, Ball State University, Muncie, Indiana 47306. —Multiple forms of adenosine deaminase were first observed and studied in many species of lower animals by Ma and Fisher. Three relative molecular sizes were observed: 200,000 100,000, 30,000, and designated as enzyme forms A, B, and C, respectively. Evidence by Ma has shown the smallest multiple form to be a monomeric unit of the largest.

Akedo, Nishihara, Shinkai, and Komatsu, have since studied similar multiple forms corresponding to the A and C forms in certain human tissues. They have reported that while normal human lung tissue contains only the A form adenosine deaminase, cancerous lung tissue contains both A and C forms.

The present study enlarges the survey of multiple forms in both normal and cancerous human tissues. Kinetic, physical, and immunological parameters are presented by way of comparison of the multiple enzyme forms extracted from various tissues. Thin-layer gel filtration and electrophoretic techniques have been applied to the study and characteristic patterns were discussed. Partial purification of the monomeric adenosine deaminase from the cerebrum has been achieved and pertinent data were reported.

¹Supported by the Little Red Door, The Delaware County Cancer Society.

Photochemical Synthesis and Destruction of Oxaziranes. M. L. DRUELINGER and S. R. LAMMERT, Department of Chemistry, Indiana State University, Terre Haute, Indiana, 47809. —The irradiation of a series

of nitrones was studied. The compounds selected for this study possessed N-aryl or N-t-alkyl groups, while the nitrone carbon was either unsubstituted (methylene) or carried a single phenyl group. The reactions were carried out in a nuclear magnetic resonance tube using a medium pressure mercury arc. In all cases the initially formed product was the oxazirane whose photochemical stability was dependent upon its substituents and the irradiation wavelength. Subsequent reaction occurred via rearrangement to give amides or fragmentation to give nitrenes and carbonyl compounds. Mechanistic studies indicate a singlet excited state for the former and a triplet excited state for the later reaction.

NOTE

Some New Approaches to Teaching Chemistry for Non-Science Majors.

LAWRENCE L. GARBER, Department of Chemistry, Indiana University at South Bend, South Bend, Indiana 46615.—If today's college student is to deal constructively with present and future social and technical problems, he must have a basic understanding of the fundamentals of science. The course described here is designed to enable the non-science oriented student to learn some of the fundamentals of chemistry but in a stimulating manner. To meet this objective, topics such as environmental pollution, polymer chemistry, chemical processes important to life, and certain aspects of drug chemistry are used to exemplify chemical phenomena. The course meets for 4 hours of lecture-discussion and 2 hours of laboratory work per week for 15 weeks.

Part I of the course emphasizes properties of atoms, electronic structure, periodic trends, oxidation numbers, simple theories of chemical bonding and the rationale behind why chemical changes take place as they do. Part I is designed to provide sufficient background for the discussion of subsequent topics. To illustrate why Part I is necessary, consider the production of sulfur dioxide which is a basic air pollutant. If the student understands electronic structure and periodic trends, the student can very easily predict that sulfur, when combined with oxygen, can have an oxidation number of plus four and thus a combustion product of sulfur will be sulfur dioxide. This type of logic can be applied to many simple, yet important, reactions that occur in pollution production and abatement.

Part II of the course emphasizes the topic of air and water pollution which obviously cannot be extremely detailed. Five main classes of air pollution; sulfur oxides and related compounds, nitrogen oxides and related compounds, hydrocarbons, carbon monoxide, and particulate matter, are included in the discussion. The student is exposed to some of the important chemical reactions that are instrumental in the production of air pollutants; chemical reactions that can be potentially harmful to life and matter; and chemical processes that might render solutions to the problems. The greenhouse effect is discussed. The factors and conditions necessary for the production of smog are emphasized.

The discussion of smog production and composition provides an excellent mechanism for the introduction of organic chemistry. The topic of

air pollution can be used to illustrate acid-base neutralization, chemical properties of acidic and basic anhydrides, pH, oxidation-reduction, colloids and colloidal properties. The discussion of water pollution very easily permits the illustration of such phenomena as ionization, electrolytes, solubility, hydrogen bonding and concentration units.

Water pollution topics include a discussion of eutrophication by phosphates and nitrates, the chemical and physical properties and biodegradability of detergents, and the essential chemical characteristics of phosphorous, nitrogen and sulfur cycles. A discussion of water hardness and the use, characteristics and effects of water softeners are easily incorporated in Part II. The topic of mercury pollution and associated controversies demonstrates readily the interrelationships of inorganic and organic chemistry.

Part III emphasizes organic and biochemistry. A discussion of polymers illustrates successfully some additional characteristics of organic compounds. A discussion of pesticides, polychlorobiphenyls and selected aspects of drug chemistry are also included. The characteristics and roles of DNA and RNA in life processes are emphasized as well as the characteristics of fats, proteins and carbohydrates.

The laboratory is designed to supplement the lecture. For 5 weeks the student investigates air and water pollution. Examples of some of the determinations are pH, color, temperature, dissolved oxygen, dissolved carbon dioxide, hardness, detergent content (ABS and LAS) and concentrations of sulfate, phosphate, nitrate, nitrite, copper, and chloride ions in local water systems. Except for temperature, color and pH measurements, Hach chemical kits, which are specifically designed for each determination, are used. The advantage of using these chemical kits, or other commercial chemical kits, is that the reagents are pre-measured, the glassware is calibrated, and the detection is simple. The amount of substance present is indicated quantitatively by either a color change or a color comparison. The detection scheme is calibrated so as to permit direct determination of concentration without the need for mathematics. Hence, the result is not masked by mathematical difficulties that the student might have. All results are collected on a data sheet. The laboratory results help to promote considerable discussion and illustrate the quality of water in the South Bend area.

In conclusion, by presenting the topics of environment pollution, aspects of drug and polymer chemistry and some of the essentials of the chemistry of life processes, important topics and concepts of chemistry can be discussed and learned by the student but in a more interesting fashion.

ECOLOGY

Chairman: CARL H. KREKELER, Department of Biology,
Valparaiso University, Valparaiso, Indiana 46383

ALTON A. LINDSEY, Department of Biological Sciences,
Purdue University, Lafayette, Indiana 47907, was elected
Chairman for 1972

ABSTRACTS

A Water Quality Study of the Tippecanoe River. PAUL T. MCKELVEY, Tippewa Technical Institute (IVTC), Lafayette, Indiana 47905.—A field study of water quality of the Tippecanoe River in north-central Indiana was conducted during June-September 1971. Surface samples were collected at 18 sites covering the 168-mile length of the Tippecanoe River from North Webster to Delphi, Indiana. Quantitative determinations were made for temperature, dissolved oxygen, total alkalinity, ortho-phosphate, nitrate-nitrogen, pH, and total coliform bacteria. Results indicated a high potential productivity due to more than adequate quantities of dissolved nutrient material. Total coliform bacteria counts were typical of small river systems flowing through both rural and urban areas.

The Howe's Rapid Biochemical Oxygen Demand Index and its Technique. ROBERT H. L. HOWE, Eli Lilly and Company Tippecanoe Laboratories, Lafayette, Indiana 47902.—The meaning of a rapid biochemical oxygen demand index and its usefulness was described. The development of a technique for the determination of this index was presented.

Stimulation of Branch Abscission in *Quercus alba* by 2-Chloroethylphosphonic Acid. WILLIAM R. CHANEY, Department of Forestry and Conservation, and A. CARL LEOPOLD, Department of Horticulture, Purdue University, Lafayette, Indiana 47907.—Abscission of young branches of a mature white oak (*Quercus alba*) was stimulated by 1 per cent and 2 per cent 2-chloroethylphosphonic acid in lanolin applied to the base of the branches. Treatments were applied 4 times at approximately 2-week intervals in the fall. The greatest stimulation of branch abscission resulted from treatment on September 11. Thirty-six per cent of control branches were shed, whereas 80 per cent of branches treated with 2-chloroethylphosphonic acid abscised. The current shoot of abscised branches was significantly shorter than the current shoot of branches retained on the tree, suggesting that the less vigorous branches were shed. Branches of juvenile trees of white oak did not abscise naturally and were not induced to abscise by treatment with 2-chloroethylphosphonic acid.

Basin Leakage Considerations in Ecological and Hydrological Studies of Experimental Watersheds. H. E. McREYNOLDS, U. S. Forest Service, 633 Wisconsin Avenue, Milwaukee, Wisconsin 53203.—In the last two decades, there has been an increase in the number of ecological or hydrological watershed studies. More often, these experiments have concerned

nutrient cycling, water-yield, and measurements of vegetative manipulations. Basically, these studies have considered the watershed to be a collecting basin with a single output point.

Too often, these watershed studies have not considered hydrologic "basin leakage" (*i.e.*, interbasin imports and exports of ground water flow). This paper explored a number of processes by which interbasin transfer can occur (non-coincidental topographic and phreatic divides; interbasin solution tubes; intercepting pre-glacial channels; seasonal variations in flow direction). It appears that the basic assumptions concerning precipitation input area and piezometric flow patterns are seldom validated in the experimental design.

NOTE

A New System for Ecological Education—SEE. JOHN W. HART, Hayes Research Foundation, Inc., and JESSIE M. TURNER, Finley School; Richmond, Indiana 47374.—Man's activities are shaping his environment; in turn, the environment he creates surreptitiously molds him. The industrial revolution initiated a speed up in man's ability to modify his surroundings. The pressures of a steadily increasing number of people, each demanding more and more goods and services are dictating the direction of movement of environmental change. That these forces are carrying us on a tidal wave in the wrong direction is particularly apparent in the developed nations of the world. We are surrounded on all sides by activities which degrade our environment—and hence our lives. Our senses are constantly insulted by noise, visual blight, dirty water, and stinking air, but man is a sensitive creature, and it is this very sensitivity which separates him from the other animals. The problem is not that man will not be able to adapt to this hostile environment. The problem is that he is doing so already, and in so doing, he is becoming a less sensitive organism. When man has finally lost his sensitivity, man has been lost. Nature may add a noble name to the list of animal failures.

Education is the last hope for creating citizens who are environmental assets, members of a citizenry attuned to its responsibility to all living things on Earth.

The system which provides the American education has for the most part given only lip service to the need for environmental training. An equally serious indictment of the system is that it has not recognized the life-centered approach to learning as an exciting way to escape today's educational doldrums. A few innovative teachers have found that students can be turned on by *involving them* in the study of their surroundings and the interrelationships therein. Unfortunately the efforts of these teachers, while not wasted entirely, are greatly diluted by the absence of any continuity of environmental learning. The one shot environmental/ecological learning experience may come at any level from kindergarten thru college, or even in that limited education after college or high school which we call "continuing".

SEE, the System for Ecological Education, was created to fill the need for an orderly, planned, life-centered approach to education which

will encompass the entire educational experience of an individual from kindergarten throughout life. SEE introduces a systematic way of developing in each child an understanding of environmental problems, their causes, solutions, and man's moral responsibility to solve them. It introduces and progressively develops concepts. At each grade level the student attains a more comprehensive understanding of each component of the environment and of the interrelationships among its physical and biological components.

SEE is envisioned as a unifying framework within which the environmental inputs of all educators may become cumulative. The system, while designed primarily for use as a K-12 instrument, can be used to develop college courses for teachers, resource majors, and students of other disciplines. It is equally adaptable for use in adult education.

OTHER PAPERS READ

Natural Areas and the Indianapolis Parks Department. GLENN PATRICK JUDAY, Department of Biological Sciences, Purdue University, Lafayette, Indiana 47907.

Response of Eyeless Cave Beetles to Light. CARL H. KREKELER, NEAL BRANDT, and BRUCE RUTLAND, Department of Biology, Valparaiso University, Valparaiso, Indiana 46383.

Calibration Techniques for Remote Sensing Measurements of Water Temperatures¹

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Purdue University, Lafayette, Indiana 47907

Abstract

Remote sensing techniques, involving optical-mechanical scanners mounted in aircraft, offer great potential for meeting some of the needs for more quantitative information on our water (and other) resources. One technique used in remote sensing allows the absolute temperature of water surfaces to be determined from several thousand feet of altitude to within 0.5 degree Centigrade. Two methods for calibration of thermal infrared scanner data were described. The accuracy of these techniques was shown. The limitations of the calibration procedures and use of airborne scanner systems were discussed.

Introduction

One recently-developed remote sensing tool is a "thermal infrared optical-mechanical scanner" or "thermal IR scanner." This instrument can be mounted in an aircraft and flown over an area of interest, thereby allowing one to obtain a temperature map of the area below the aircraft.

Calibration Techniques

To make thermal IR scanner data meaningful, the relative radiance values (as measured by the scanner) must be related to the true temperature of the area being sensed, or target area. Two quite different techniques are possible. These are 1) the correlation method, and 2) the internal calibration techniques.

The correlation method is relatively simple, but does have some serious drawbacks. Variations in tone on the thermal IR imagery correspond to relative differences in the radiant temperatures of the scene imaged. Therefore, if the actual temperature of the various objects on the earth's surface can be determined, it is possible to correlate these measurements with density values of the imagery to those points on the ground. To use this technique for water studies, temperature measurements must be obtained for a number of locations on the river or lake at the time the aircraft passes overhead. Later, these measurement points are located on the thermal IR imagery and the density of the film at these points is measured. In this way, correlation is possible between actual temperature of the water at a few points and the film density at these same points. Intermediate film density values can then be interpolated.

The major drawbacks to this technique are that, 1) it takes only a few minutes to obtain the scanner data via airplane, but it may take

¹The work described in this paper was supported by the National Aeronautics and Space Administration, under Grant #NGR 15-005-112.

²Associate Professor, Department of Forestry and Conservation, and Graduate Student, Geosciences Department, respectively.

several hours to obtain surface temperature measurements, during which time the water temperature may have changed significantly, and 2) development of the film on which the data are recorded is subject to many possible variations in chemical processing which can sometimes cause tonal differences to not have a linear relationship.

For several years, the correlation method was the only technique available, due to limitations in the thermal IR scanning equipment. Relatively recent developments in scanning equipment have allowed researchers to devise an "internal calibration" technique, which, with two assumptions, allows the actual water temperature to be determined directly from the scanner data, and no surface observations of temperature are necessary (other than to verify the reliability of the technique). One assumption is that the radiation characteristics of water in the thermal IR region approximate those of a black-body radiator (which is defined as an object that will completely absorb all frequencies of radiation incident upon it). Water departs only 2-3% from being a perfect black-body radiator in these thermal IR wavelengths (1). The second assumption is that atmospheric attenuation has relatively little effect on the amplitude of the signal received at the scanner. This assumption is based on the fact that the 8.0-13.5 μm region of the electromagnetic spectrum is in an atmospheric window (where absorption by the various components of the atmosphere is minimal), and also that most of the small amounts of energy absorbed by water vapor or other atmospheric constituents would be reradiated by the water vapor since water acts as a black-body in this portion of the spectrum, thereby allowing only extremely small net losses of radiated energy between the water surface and the aircraft scanner. Previous work has indicated that these assumptions appear to be reasonable (3).

The data used by LARS in this work was obtained by a multispectral scanner, owned and operated by the University of Michigan, and mounted in a C-47 aircraft (4). This scanner has been modified to accommodate two reference black-bodies which are viewed by the scanning mirror during each revolution. These reference sources are referred to as calibration plates, and are temperature controlled, one plate being cooler than the other, in approximately the same range of temperatures of the earth surface features over which the aircraft is flying (2).

Methods

The objective of this study was to determine the reliability of the temperature calibration procedures using data collected over the Wabash River at two different times of the year, and under somewhat different weather conditions. On June 30, 1970, data were collected from an altitude of 3,000 feet at 10:45 AM, with good clear weather conditions, while on August 13, the altitude was 2,000 feet, with somewhat hazy weather conditions and at 3:47 PM.

Using the data logs on the calibration plate temperatures, the data were calibrated for radiant temperature and gray scale maps were produced. The different temperature ranges were depicted by different

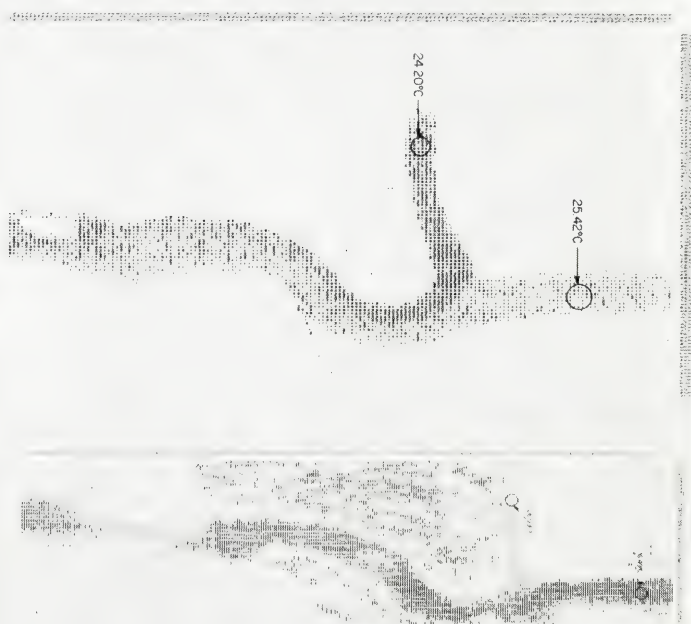
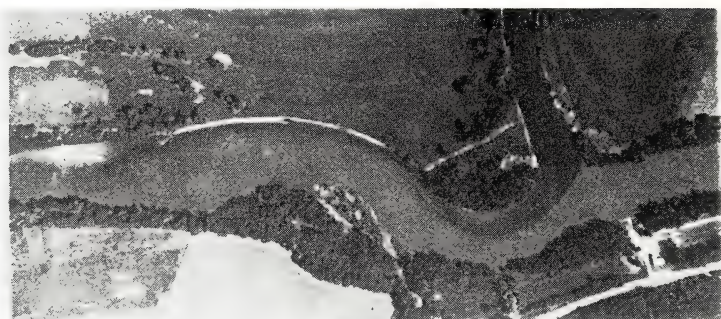


FIGURE 1. An aerial photograph and computer printouts of calibrated thermal IR scanner data, collected June 30 from 3,000 feet (center) and August 13 from 2,006 feet altitude (bottom). The junction of the Tippecanoe River (from top) and Wabash River (from right) is shown. Note that the June 30 data shows that the water in the Tippecanoe is cooler than the Wabash, while the opposite is true for the August 13 data. The August data also shows much of the land area at the same apparent temperature range as the water.

Symbol Key. Temperature in °C.

June 30. (blank): 24.0 or lower, (M): 24.0-24.9, (=): 24.9-25.2, (—): 25.2-25.5, (.): 25.5-26.0, (blank): 26.0 or higher.

August 13. (blank): 25.4 or lower, (M): 25.4-25.9, (=): 25.9-26.2, (—): 26.2-26.5, (blank): 26.5 or higher.

computer symbols. Only those temperature ranges that corresponded to the river were displayed. A blank, or no symbol, was used for all other temperatures.

Results

The junction of the Tippecanoe River (from left) with the Wabash river (from top) is illustrated in Figure 1. On June 30, the actual water temperatures, as measured from the boat, showed very good correlation with the temperatures indicated by the calibrated scanner data. Note that the Tippecanoe River was approximately 1.2°C cooler than the Wabash, and that this temperature difference persisted for a considerable distance downstream. On August 13, the Tippecanoe was relatively warmer than the Wabash, but the calibrated scanner data indicates about 0.8°C lower temperatures than were actually measured. Since the August data were obtained under hazy conditions, this could be the cause of the calibration error, and would indicate that additional work is required to obtain a fully reliable calibration technique.

Note that on the June 30 data only the river areas are shown on the printouts, indicating that only the water occupied the temperature ranges indicated, but on August 13, considerable land areas were emitting the same amounts of energy as the water. This indicates that if one is to produce temperature maps showing only the water areas, a layered classification scheme will need to be developed whereby one first identifies all water areas, and then maps the temperature characteristics of the water only.

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Natural Areas in the Beech-maple and Maple-basswood Forest Regions of Nine States

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Abstract

Four hundred and eighty-two natural areas of biological or geological interest are now reported within the Beech—maple and Maple—basswood Regions of the 9 states which include these forest regions.

In Indiana, 8 forested natural areas and 17 tracts of other types were previously unpublished. Of the former, the Womer Tract, Bryan Memorial Preserve and Burgdorf Woods are described briefly.

In 1969-70 the National Park Service commissioned 5 theme-studies at as many ecological laboratories in the eastern and central United States, to obtain data from secondary sources for later use in designating many new National Natural Landmarks. The basis for organizing this overall survey was the forest region map inside the back cover of Braun (1950). Our contribution (3, 7) was a study based largely on correspondence, interviews and published sources, of the two forest regions dominated by sugar maple. The Maple-basswood region is confined to parts of Illinois, Wisconsin and Minnesota, whereas Beech-maple occurs in New York, Pennsylvania, Ohio, Michigan, Illinois, Wisconsin, and Indiana. The literature cited consists of 204 titles.

The concept of natural areas and the rationale and techniques of their description and preservation were presented in references 2, 4, 5, 6, 7, 8 and 9. W. B. Barnes has written popular descriptions in "Outdoor Indiana" magazine of most of the 17 areas in the state system of nature preserves, of which he is the Director.

We acknowledge with thanks the information about "new" Indiana stands received from William B. Barnes, Mrs. Thomas E. Dustin and Floyd Swink. Among the multitude who helped provide data for other states condensed into Table 1 we are especially indebted to Drs. Grant Cottam, J. Arthur Herrick, Kenneth S. Erdman and William Tans.

Summary for the Two Entire Regions

A table of data giving only one line for each of 482 tracts of land was prepared, but proved too voluminous for publication. A more generalized summary, given in Table 1, categorizes areas by the single natural feature of special interest, usually the one constituting the chief reason for considering the site worth preserving. Those of the 9 states for which Table 1 shows few tracts are not deficient in natural areas if the whole

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state were considered, but, rather, are states having little acreage of the two of Braun's vegetational Regions that we studied for this project. The larger tracts are often state parks, wildlife areas or metropolitan parks, that possess a great diversity of natural interest in addition to recreational facilities. These are listed as "large, varied" areas. Since our bias as ecologists is toward communities, there is much more of geological interest encompassed in the sites than is reflected by the table. (Practically all the Beech-maple stands occupy land that has been glaciated, for example). The line for "Other geology" includes mostly waterfalls. The tracts considered under "Gorges, bluffs" very often contain relict hemlock colonies. Caves and archaeological sites were omitted. Even though several or many natural features were present, a site is listed only once; therefore, the numbers and totals are significant.

TABLE 1. *Number of natural areas, classed by their chief feature and by state, in only the portions of the state inside the Beech-maple and Maple-basswood Regions of Braun (1). Indiana data are based on Lindsey, Schmelz and Nichols (6) and the present study.*

Types	Ind.	Ia.	Ill.	Wis.	Minn.	Mich.	Ohio	Pa.	N.Y.
Beech-maple	23	—	—	13	—	6	12	2	1
Oak-hickory	16	—	3	5	—	4	11	—	—
Mixed Woods	14	—	10	19	2	3	21	—	1
Maple-basswood	—	—	1	15	4	—	—	—	—
Hemlock-hardwoods	—	1	—	10	—	—	9	1	2
Oak savanna	1	—	—	2	1	—	3	—	—
Flood Plain	2	1	2	4	1	4	3	—	—
Swamp Forest	5	—	1	4	—	4	13	2	2
Bog or Fen	6	—	6	9	1	3	14	10	3
Marsh	—	—	—	2	—	1	3	—	1
Varied Wetlands	—	—	1	1	—	3	9	—	1
Lake, pond	4	—	—	1	1	2	12	1	—
Sand dunes	1	—	2	3	—	1	2	—	—
Prairie	10	—	6	9	3	4	—	—	—
Glacial geology	—	—	—	1	—	—	3	—	2
Gorge; bluff	5	1	1	7	—	—	13	2	—
Other geology	3	—	—	1	—	—	4	—	—
Large, varied	2	—	1	3	2	3	9	2	1
Wildlife refuge	—	—	1	1	—	1	4	—	4
Heronry	1	—	—	—	1	—	2	—	1
TOTALS	93	3	35	110	16	39	147	20	19

Nearly all the good natural areas and nature preserves in Indiana had been described in Lindsey, Schmelz and Nichols (6). Additional Indiana tracts reported to the NPS by the present authors are also included in Table 1.

Indiana Areas Previously Unpublished

Further details on "new" Indiana natural areas are given in Table 2 for forests and Table 3 for tracts where the principal feature is not the tree community, if any.

The largest and most diversified of the new Indiana nature preserves is that donated to the State System of Nature Preserves in 1970 by Mr. and Mrs. John Womer of Chicago. It is 4 miles south of Chesterton and 1 mile east of Rt. 49 on Rd. 750 N. It is in two parts lying a quarter-mile apart on rough land of the Valparaiso moraine. The upland supports a disturbed oak woods, and, in much of the north 80, early lumbering left open conditions now showing early successional stages with scattered small trees and shrubs. About one-third of shallow Carlson Pond lies within the southern 80. There are also 2 small kettle ponds, and a former pond nearly as large as Carlson Pond is now a large depression supporting buttonbush and willows. In the southern part of the south 40, about 10 acres of a mixed forest appear to be essentially undisturbed except for an old woods road.

The Eunice Hamilton Bryan Memorial Nature Preserve is also within the state system under Director William B. Barnes. It was bequeathed by the late Henry R. Smith of Rossville, and named in honor of the mother of Mrs. Smith. The tract was previously known as Smith Woods. The area is notable for many large old trees and includes several permanent ponds which, in April, occupy about 6 of the 29 acres. The soils are Crosby and Brookston. Table 4 gives data on vegetational attributes of the oak-hickory stand on high ground away from the pond edges, and indicates that the major dominant, white oak, had more than double the importance value of shagbark hickory. Irrespective of species, the average acre of the 4 acres (contained within the 0.2 acre sampling strips) showed 101 individual trees over 4 inches diameter at breast height. These trees on the average acre had 117 feet basal area, which is high for Indiana forests. In the 4 acres within the sampling strips, species that had one or more individuals in the 30-inch or larger size class were white oak, red oak, swamp white oak, beech and bur oak. There were 29 such trees sampled, including 18 white oaks, ranging up to the 46-inch size class which had two individuals. The largest red oak was 38 inches and the only swamp white oak measured 42 inches.

Burgdorf Woods, in the extreme southwest corner of Vanderburgh County, has only 10 acres of large, old-growth timber, which is surrounded by woods of lesser quality. It represents the Oak-hickory type, with stratum ranks as follows for trees exceeding 4 inches dbh—red oak (including Shumard's) 7, black oak 6, tulip tree 6, white oak 5 and white ash 5. The species with some stems exceeding 30 inches dbh were Shumard's red oak with 13 such stems, black oak had 10, tulip tree 7, white oak 5, white ash 3, and sugar maple, sycamore and bitternut hickory with one each. In all reproduction strata, sugar maple dominated substantially. Twenty-three species had stems exceeding 22 inches dbh. Of the 142 larger trees measured in the 10 acres, 95 exceeded 22 inches.

TABLE 2. Forested natural areas in Indiana, previously unpublished. BM indicates Beech-maple type, OH-hickory, and FP is flood plain stand type.

Type	Acres	Quadrangle	Twp. & Range	Section	Owner
Burgdorf Wds. (Vanderburgh Co.)	10	W. Franklin	7S, 12W	18, NW	Mrs. P. T. Burgdorf
Bryan Memorial (Clinton Co.)	29	Frankfort	25N, 2W	13, NE $\frac{1}{4}$ of NE $\frac{1}{4}$	Div. Nature Pres.
Cameron Wds. (Steuben Co.)	28	Metz	36N, 15E	8 NW $\frac{1}{4}$ NW $\frac{1}{4}$	W. Bennett
Klingler Wds. (Allen Co.)	29	Garrett	32N, 12E	3 SW $\frac{1}{4}$ NW $\frac{1}{4}$	R. H. Klingler
Miller Tract (Allen Co.)	80	Cedarville	32N, 12E	11 NW $\frac{1}{4}$	Alpha Miller
Scout Ridge (Monroe Co.)	15	Hindustan	10N, 1E	3 W $\frac{1}{2}$ Ctr.	Nature Pres.
Seaburg Wds. (Noble Co.)	17	Albion	34N, 9E	27 NW $\frac{1}{2}$	H. Madison
Womer Tract (Porter Co.)	160	Chesterton	36N, 5W	29 & 32	Div. Nature Pres.

TABLE 3. Non-forest natural areas in Indiana previously unpublished.

Acres	Quadrangle	Twp. & Range	Section	Owner
Arethusa Bog	75	Angola W.	19 SE & SW $\frac{1}{4}$	Eugene Cope
Binkley E. Bog	70	"	30 NE & NW $\frac{1}{4}$	Mr. Binkley
Hickman Lk. Bog	—	Ege	31, 36, 38	A. M. Geiger
Koester Bog	25	Ormas	28, E $\frac{1}{2}$	Marie Koester
Mill Creek Fen	—	Stillwell	32, SW $\frac{1}{4}$	—
Greenwood Pond	25	Auburn & Cedarville	33, SESE	H. E. Greenwood
No name Pond	—	New Carlisle	34, SWSW	—
Garretson Heronry	30	Largo	7 NW $\frac{1}{4}$, N $\frac{1}{2}$	L. A. Garretson
Beaver Lk. Prairie	640	Enos	30	Div. Nature Pres.
Haskell Prairie	—	Wanatah	4 or 5	Monon R.R.
Lake Co. Prairies ¹	—	—	8 SWSE	Various
Lake Pleasant Marl Prairie	95	—	17 NENW, NWNE	Ben Collins
Monon R.R. Prairie	—	Angola W.	13, 14, 18	P. R. & State
Shallow Lk. Prairie	140	Monticello N.	Linear	Mr. Rude
Trail Creek Site	—	Orland	7	Mich. Twp.
Devil's Hollow	60	Mich. City E	35, SW $\frac{1}{4}$	R. L. & M. L. Mellen
Shanty Falls	40	Arcola	19, SE $\frac{1}{4}$	Don Stouffer
		Wabash	20, SW $\frac{1}{4}$	
			Reserve 55	

¹Includes mostly small remnant areas of prairie close in to industry and railroads, such as Cook Prairie (St. John village), Miller Dune tract, Peponon Prairie (Gary, W), and Tolleston Area (Hammond). Specific Locations in (3) and (7).

TABLE 4. *Vegetational Attributes¹ in the high ground, old growth stand at Bryan Woods, Clinton County, Indiana.*

Species	D ₂	D ₃	B ₂	B ₃	F ₃	V ₃
White oak	13.5	13.3	51.5	44.1	13.6	23.6
Shagbark hickory	13.2	13.0	8.8	7.5	14.1	11.5
White ash	14.5	14.2	7.8	6.6	13.2	11.3
Ironwood	14.0	13.7	2.1	1.8	12.5	9.4
Basswood	12.2	12.0	2.6	2.2	8.5	7.6
Beech	4.5	4.4	7.5	6.4	4.7	5.2
Sugar maple	5.2	5.1	4.3	3.7	5.5	4.8
Red oak	4.2	4.1	16.9	14.5	5.5	4.7
American elm	5.2	5.1	.8	.74	5.9	3.9
Pignut hickory	4.0	3.9	2.6	2.2	4.0	3.4
Bur oak	1.2	1.2	3.3	2.8	1.31	1.80
Wild cherry	2.0	1.97	.25	.21	2.22	1.47
Walnut	1.5	1.48	1.34	1.15	1.56	1.40
Swamp white oak	.2	.25	2.5	2.17	.30	.91
Blue beech	1.0	.98	.13	.11	1.31	.82
Silver maple	1.0	.98	.96	.82	.65	.80
Sycamore	.50	.49	1.00	.86	.65	.67
Red maple	.75	.74	.42	.36	.91	.67
Bitternut hickory	.50	.49	.79	.68	.65	.61
Hackberry	.50	.49	.10	.08	.65	.41
Flowering dogwood	.50	.49	.05	.04	.65	.39
Sassafras	.25	.25	.27	.23	.30	.26
Tulip tree	.25	.25	.08	.07	.30	.21
Pin oak	.25	.25	.05	.04	.30	.20
Red elm	.25	.25	.04	.03	.30	.19
TOTALS	D ₉ =101		B ₉ =117			

¹Based on trees 4 inches or more dbh. Twenty sampling strips (each 400 feet long and one-fifth acre area) were laid out, totalling 4 acres in area. D₂ symbolizes density per mean acre, D₃ is relative density, B₂ is basal area per acre, B₃ relative basal area, F₃ is relative frequency and V₃ importance value.

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Importance Per Cent Values of a Browsed Southeastern Indiana Forest¹

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Abstract

White ash (*Fraxinus americana*), hop hornbeam (*Ostrya virginiana*), red oak (*Quercus rubra*), yellow poplar (*Liriodendron tulipifera*) and beech (*Fagus grandifolia*) exhibited importance per cent values of 49.4, 37.4, 28.6, 26.5 and 20.3 respectively, for a 10-acre forest located on the Brookville Ecology Research Center in southeastern Indiana. These values represented 54 per cent of the total tree species sampled within an area which had recently been released from browsing. White ash, hophornbeam, sugar maple (*Acer saccharum*), redbud (*Cercis canadensis*) and honey locust (*Gleditsia triacanthos*) were represented by a high density of seedlings (< 1 inch Diameter Breast Height), while canopy species of yellow poplar, red oak, beech and bitternut hickory (*Carya cordiformis*) were poorly represented in smaller size classes.

Introduction

Systematic sampling of a forest area following removal of cattle reveals much quantitative information regarding secondary succession. Cattle prefer tender grasses, forbs and herbs, in addition to shoots and tree seedlings (6). Effects of browsing also include such physical changes as compaction of soil, promotion of erosion, damage to root systems, litter removal, and general degradation of the natural seedbed conditions (6). Although several recent studies have been concerned with deciduous forests in this region of the midwest (2, 7, 8, 11), only limited information is available regarding the effects of browsing on the composition of forest communities. Thus far, only the study by Day and DenUyl (3) has provided specific information on browsing pressures by cattle in Indiana forests. We attempted to better measure and evaluate the effects of browsing on the composition of a mature deciduous forest community located in southeastern Indiana.

Study Area

The study area is located in Union County, south of Liberty, Indiana, specifically in the NW 1/4, Sec. 22, T11N, R2W on the Fairfield, Indiana, Quadrangle. The site is included within the Brookville Ecology Research Center, administered by Miami University, Oxford, Ohio, and Earlham College, Richmond, Indiana. The actual study area was comprised of 10 acres of forest located on the 140-acre research station leased in 1969 from the Army Corps of Engineers. The remaining 130 acres had been used annually for agricultural crops (*e.g.*, corn, soybeans, etc.) until the spring

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of 1970. The total area had since been left undisturbed to proceed through secondary succession. The 10-acre forest area had been periodically grazed by a herd of cattle for at least the past 8-10 years. This herd ranged in size from 6-15 cattle and was permitted to graze this area periodically during the spring and summer months out of each year. Cattle were permanently removed from the study site approximately 3 months before sampling was initiated.

The forest is located on a gradual-moderate slope with a total relief of 140 feet from the highest to lowest points and lies just north of the Wisconsin glacial boundary. The physiography is defined as Dearborn Upland, a dissected plateau north and west of the Ohio River with streams in deeply incised valleys (12). Soils for this area have been reported by Alfred, Ulrich and Zachary (1). Climatic conditions for this area have also been previously described by Schaal (10).

Methods and Materials

The nested quadrat method was used for sampling the vegetation (9). This method provides adequate sampling within a community of varying plant sizes. Ten nested quadrats, each 66 feet by 66 feet (0.1 acre each), were placed randomly within the 10-acre forest area. Thus, 10 per cent of the total forest area was sampled. Each 0.1-acre quadrat contained one 6.6 feet by 66.0 feet plot (0.01 acre) and two 6.6 feet by 6.6 feet square plots (0.001 acre or 1 milacre).

During October, 1970, all trees greater than 3 inches in diameter breast height (dbh) were tallied in the 0.1-acre plots. All saplings over 12 inches in height and less than 3 inches dbh were tallied in the 0.01-acre plot and all seedlings less than 12 inches in height were tallied in each milacre plot. The number of trees per acre, relative density, frequency of occurrence, relative frequency, total basal area, relative cover, and importance per cent values were computed (9). Frequency distribution of all species was computed by size classes. Diameter measurements of all trees were rounded to the nearest inch. Basal area for each species was calculated using a conversion table (9).

Results and Discussion

White ash (*Fraxinus americana*), hop hornbeam (*Ostrya virginiana*), red oak (*Quercus rubra*), yellow poplar (*Liriodendron tulipifera*), and beech (*Fagus grandifolia*) were clearly dominant, having importance per cent values of 49.4, 37.4, 28.6, 26.5, and 20.3, respectively. These species constituted 54% of all tree species sampled. (Table 1). Hop hornbeam, usually a mid-canopy species in white ash-yellow poplar-red oak associations (5) was the most frequently encountered species. White ash, red oak, yellow poplar, and beech, the major canopy species, had relative cover values of 20.7, 18.9, 13.6, and 12.8%, respectively, representing approximately 66% of the total cover (see Table 1).

The above-mentioned dominant trees (i.e., white ash, hop hornbeam, red oak, yellow poplar, and beech) exhibited maximum dbh measurements of 18, 9, 34, 31, and 23 inches, respectively. Other large trees in-

TABLE 1. Summary of plant community parameters based on all trees over 3.0 inches dbh.

Tree species ¹	No. trees per acre	Relative density (A)	Frequency	Relative frequency (B)	Basal area per acre ft. ²	Relative cover (C)	Importance per cent value (A+B+C)
1. Fa	27	16.9	8	11.8	20.9	20.7	49.4
2. Ov	34	21.2	7	10.3	6.0	5.9	37.4
3. Qr	6	3.8	4	5.9	19.1	18.9	28.6
4. Lt	16	10.0	2	2.9	13.7	13.6	26.5
5. Fg	5	3.1	3	4.4	12.9	12.8	20.3
6. C cor	10	6.2	5	7.4	4.1	4.1	17.7
7. Ur	9	5.6	6	8.8	2.0	2.0	16.4
8. As	6	3.8	5	7.4	4.3	4.3	15.5
9. C can	13	8.1	3	4.4	1.7	1.7	14.2
10. Ps	4	2.5	4	5.9	5.8	5.7	14.1
11. C car	9	5.6	5	7.4	1.0	1.0	14.0
12. C oc	5	3.1	4	5.9	1.3	1.3	10.3
13. C cr	5	3.1	3	4.4	0.6	0.6	8.1
14. Sa	2	1.3	2	2.9	1.5	1.5	5.7
15. Mp	2	1.3	2	2.9	0.2	0.2	4.4
16. Po	1	0.6	1	1.5	1.8	1.8	3.9
17. Gt	2	1.3	1	1.5	1.0	1.0	3.8
18. Q mu	2	1.3	1	1.5	0.7	0.7	3.5
19. C ov	1	0.6	1	1.5	1.2	1.2	3.3
20. Fq	1	0.6	1	1.5	1.1	1.1	3.2
Totals	160	100.0		100.2	100.9	100.1	300.3

¹Full nomenclature (5) is as follows: 1. *Frazinus americana* L., White ash; 2. *Ostrya virginiana* (Mill.) K. Koch, Hop hornbeam; 3. *Quercus rubra* L., Red oak; 4. *Liriodendron tulipifera* L., Yellow poplar; 5. *Fagus cordifolia* Ehrh., Beech; 6. *Carya cordiformis* (Wang.), K. Koch, Bitternut hickory; 7. *Ulmus ruba* Muhl., Slippery elm; 8. *Acer saccharum* Marsh., Sugar maple; 9. *Cercia canadensis* L., Redbud; 10. *Prunus serotina* Ehrh., Black cherry; 11. *Carpinus caroliniana* Walt., Blue beech; 12. *Celtis occidentalis* L., Hackberry; 13. *Crataegus crux-galli* L., Hawthorn; 14. *Sassafras albidum* (Nutt.), Nees, *Sassafras*; 15. *Maclura pomifera* (Raf.), Schneider, Osage orange; 16. *Platanus occidentalis* L., Sycamore; 17. *Gleditsia triacanthos* L., Honey locust; 18. *Quercus muehlenbergii* Engelm., Chinkapin oak; 19. *Carya ovata* (Mill.) K. Koch, Shagbark hickory; 20. *Frazinus quadrangulata* Michx., Blue ash.

cluded black cherry (*Prunus serotina*), sugar maple (*Acer saccharum*), sassafras (*Sassafras albidum*), shagbark hickory (*Carya ovata*), and bitternut hickory (*Carya cordiformis*), with maximum dbh measurements of 18, 17, 16, 15, and 12, respectively. Such major canopy species as red oak, beech, bitternut hickory, yellow poplar, and black cherry were not well represented in the small size classes (< 5.0 inches dbh), nor in the seedling class. The paucity of reproduction of the major canopy species may be due to the influence of livestock. Species dominating the mid-canopy were redbud (*Cercis canadensis*), slippery elm (*Ulmus rubra*), sugar maple, hawthorn (*Crataegus crux-galli*), paw-paw (*Asimina triloba*) and wafer ash (*Ptelea trifoliata*).

Seedlings present were mainly white ash (78,500/A), hop hornbeam (54,000/A), redbud (9,500/A), honey locust (*Gleditsia triacanthos*) (8,500/a) and sugar maple (6,000/A). Slippery elm and blue ash (*Fraxinus quadrangulata*) exhibited a seedling density of 1,500/A each. Five taxa with 500/A seedlings each were sassafras, blue beech (*Carpinus caroliniana*), osage orange (*Maclura pomifera*), chinkapin oak (*Quercus muehlenbergii*), and sycamore (*Platanus occidentalis*).

In an earlier study concerned with browsed Indiana forests Day and DenUyl (3) reported that seedlings of white ash, sugar maple, and slippery elm were selected by cattle under all browsing intensities, whereas, under light to moderate browsing (*i.e.*, 10 or more acres per cow), red oak, beech, sassafras, bitternut hickory, sycamore, honey locust, and black cherry were selected. They also reported that seedlings utilized under heavy to very heavy browsing conditions (*i.e.*, less than 4 acres per cow) included shagbark hickory, hawthorn, blue beech, hophornbeam and paw-paw. Comparing the results of Day and DenUyl (3) to those of this study, it would appear that this forest had been under moderate browsing stress due to the paucity of red oak, beech, bitternut hickory, and black cherry seedlings.

DenUyl, Dillen and Day (4) reported that white ash, slippery elm, and sugar maple were found to be prolific seed producers. This, perhaps, helps to explain much of the data in the present study, in which white ash, slippery elm, and sugar maple were represented by 27, 9, and 6 mature trees per acre, respectively, as well as each exhibiting an abundance of small seedlings.

In summary, the disturbance (browsing) of this forest community was clearly reflected by an absence of reproduction (seedlings) of over 50% of the dominant canopy species. It appears that certain dominant species based on importance per cent values (*e.g.*, red oak, yellow poplar, and beech) were adversely affected by browsing. It also appears that white ash and hop hornbeam are able to maintain their dominance even under moderate browsing stress. The reason for this browsing selection factor awaits further investigation.

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Contents of Southern Indiana Wild Turkey Droppings

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Abstract

Food item volume percentages were determined of the contents of 207 adult and 84 juvenile wild turkey droppings collected over 4 seasons.

Material found in order of decreasing volume percentages was Gramineae seeds; soil and organic debris (including grit); unidentified plant material (leaves and stems); insect parts; various seeds of *Quercus* sp., *Cornus* sp. and *Smilax* sp.

The Gramineae constitutes a large portion of the diet over all seasons with the large seeds from small woody species being important only in the fall and winter.

Insects probably are not a large part of the diet of either adult or juvenile wild turkeys.

Introduction

The eastern wild turkey (*Meleagris gallopavo silvestris* Vieillot) disappeared from northern Indiana by 1870 and from southern Indiana by 1900 (2, 4, 7). The regrowth of forests and the increased protection due to hunting laws and management programs has allowed the successful re-establishment of this species in Indiana. In 1956, wild turkeys from Arkansas were released on the Crane Naval Ammunition Depot in Martin County and later releases of wild turkeys from Missouri were made in other parts of southern Indiana between 1962 and 1966. Wild turkey populations are now established at Brown County State Park, Clark State Forest (Clark, Scott and Washington Counties), Harrison County Turkey Management Area in the Harrison-Crawford State Forest, Lost River Area of Hoosier National Forest (Martin and Orange Counties), Mogan Ridge Turkey Management Area of the Hoosier National Forest (Perry County), Owen-Putnam State Forest (Owen and Putnam Counties), and Pike State Forest (Pike County).

Indiana wild turkey management programs may extend the present range. Markley (8) and Webb (11) have noted that the shortage of food may be a factor limiting wild turkey populations. Food habits of the re-introduced wild turkey in Indiana have not been studied. These data may be used by game biologists in future management programs.

This study concerned the food habits of the wild turkey in southern Indiana. Dropping analysis was used to determine the food habits because an adequate sample of crop and gizzard contents was unavailable for analysis due to the low populations and minimal hunting take.

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Methods and Materials

Wild turkey droppings were collected by Indiana game biologists in unglaciated portions of Clark, Harrison, Perry, and Monroe Counties. Sex and age of the wild turkeys producing the droppings were determined according to dropping size and shape (1, 6). Contents of droppings were separated and identified following procedures outlined by Korschgen (5) and Eaton, Moore, and Saylor (3). All remains from a given season and age class were pooled to obtain total volume. Food item volume percentages were then based on the total sample volume (6, 9).

Results

A total of 291 wild turkey droppings (collected between January 1968 and January 1971) was examined in this study. Food item volume percentages of the dropping contents are listed by season for adults in Table 1 and for juveniles in Table 2.

Plant seeds from the Family Gramineae constitute a major portion of the diet throughout the year for both juvenile and adult birds. Plant

TABLE 1. *Food items in 207 droppings of adult wild turkeys listed by seasonal volume percentages and dates collected.*

Food Items	Volume Percentages			
	Spring (Mar.-May) n=28	Summer (June-Aug.) n=6	Fall (Sept.-Nov.) n=64	Winter (Dec.-Feb.) n=109
Soil & Debris	12.3	—	46.0	48.2
Gramineae (several genera)	50.0	83.5	59.5	3.1
Undetermined Plants parts	33.3	13.9	1.7	1.7
Insect Parts	1.0	0.4	0.3	0.6
Grit	1.5	1.6	12.3	5.8
<i>Quercus</i> sp.	0.3	0.01	0.7	13.0
<i>Cornus</i> sp.	0.9	0.3	0.7	11.9
<i>Pinus</i> sp.	0.01	0.01	—	0.3
<i>Fraxinus</i> sp.	—	—	—	0.01
<i>Cercis canadensis</i>	—	—	0.01	—
<i>Rosa</i> sp.	—	—	—	0.01
<i>Smilax</i> sp.	0.8	0.1	14.9	13.0
<i>Rhus radicans</i>	0.8	—	0.9	1.1
Misc. materials each less than 0.01 per cent volume	0.1 ¹	0.4 ²	0.2 ³	1.2 ⁴

¹*Cyperus* sp., Labiatae.

²*Lonicera* sp., *Carex* sp.

³*Physalis* sp., *Solanum* sp., *Ranunculus* sp., *Lonicera* sp., *Sida* sp., reptile scales and bird feathers.

⁴*Vitis* sp., *Lespedeza* sp., *Lonicera* sp., *Rumex* sp., *Sida* sp., reptile scales, bird feathers and mammal hair.

material of undetermined origin was second in volume percentage over all seasons for both age classes, with soil and organic debris present in large quantities as well. This organic material is probably partially digested plant seeds, undigested cellulose and insect exoskeletons.

TABLE 2. Food items in 84 droppings of juvenile wild turkey listed by seasonal volume percentages and dates collected.

Food Items	Volume Percentages			
	Spring (Mar.-May) n=3	Summer (June-Aug.) n=1	Fall (Sept.-Nov.) n=39	Winter (Dec.-Feb.) n=41
Soil & Debris	—	—	50.8	25.9
Gramineae (several genera)	91.5	93.5	3.7	10.7
Undetermined Plant parts	4.2	0.01	8.9	18.6
Insect Parts	2.7	0.01	0.6	2.1
Grit	1.7	—	3.3	3.0
<i>Quercus</i> sp.	—	—	0.5	4.7
<i>Cornus</i> sp.	—	—	8.8	1.3
<i>Pinus</i> sp.	—	—	—	0.3
<i>Fraxinus</i> sp.	—	6.4	—	—
<i>Smilax</i> sp.	0.1	—	19.9	8.0
<i>Rhus radicans</i>	—	—	2.9	6.2
<i>Lonicera</i> sp.	—	—	1.1	—
<i>Lespedeza</i> sp.	—	—	0.1	0.3
Misc. materials each less than 0.01 per cent volume	—	—	0.04 ¹	0.04 ²

¹*Desmodium* sp., reptile scales.

²*Desmodium* sp., beetle feces, and reptile scales.

Seeds from *Cornus* sp., *Quercus* sp. and *Smilax* sp. are most abundant in the droppings collected during the fall and winter, and appear to constitute a large part of the diet during those months.

Insects are not a significant part of the annual diet of adult Indiana wild turkeys, but appear to be slightly more important to juvenile birds. Schorger (10) reports that insects form the most important food of young wild turkeys, but this study indicated that few insects are ingested by juveniles.

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ENGINEERING

**Chairman: ROBERT L. SWAIM, Department of Aeronautical-
Astronautical Engineering,
Purdue University, Lafayette, Indiana 47907**

**ROBERT L. SWAIM, Purdue University, was re-elected
Chairman for 1972**

ENTOMOLOGY

Chairman: ROBERT E. DOLPHIN, U. S. Department of Agriculture
Entomology Research Division, Vincennes, Indiana 47591

CLAUDE F. WADE, Department of Natural Resources,
113 State Office Building, Indianapolis, Indiana 46203
was elected Chairman for 1972

ABSTRACTS

Insects and Other Arthropods of Economic Importance in Indiana During 1971. R. W. MEYER and J. V. OSMUN, Department of Entomology, Purdue University, Lafayette, Indiana 47907.—The abundance and activity of insects and other arthropods that were important to growers of foods, fibers, or animal products, or were destructive of stored products, or were annoying to man in the state of Indiana during 1971 were reviewed, together with notes on non-economic arthropods that were of interest.

Survival of Odonata Naiads Through Drought and Freezing. B. ELWOOD MONTGOMERY, Department of Biology, Frostburg State College, Frostburg, Maryland.—Living Odonata naiads were found in a "dry" pond in late October (October 28, 1968). They were in damp situations—masses of fine roots or accumulations of debris on the exposed bottom surface. The natural environment was restored within a few days of the initial observations by fall rains.

During the following winter an examination of the pond revealed that all water was frozen, but the bottom surface immediately beneath the ice was not.

No naiads could be recovered from the pond following the fall rains which resulted in extensive marshy margins so that the central area of the pond could not be approached. Sampling was difficult in the spring for the same reason. However, naiads of Zygoptera (none of which had been found the previous fall), Aeshnidae and Libellulidae were collected. These were of such age that development from the egg during the spring appeared extremely improbable. Thus, the naiads must have survived the elimination of all water from their habitat twice—once through drought and, then, by freezing.

Myiasis in Delaware County, Indiana, 1971, with a Confirmed Case of Infestation by *Wohlfahrtia vigil* (Walker) (Diptera: Sarcophagidae). R. E. SIVERLY, Department of Physiology and Health Science, Ball State University, Muncie, Indiana 47306.—Two fly larvae were removed from the leg of a rabbit on July 25. One of these larvae was reared to the adult stage. The pupal period required 12 days. The adult was identified by the U. S. National Museum as *Wohlfahrtia vigil* (Walker).

Wohlfahrtia is known as a specific myiasis producer, and the larvae are reported to invade unbroken skin in children, as well as in young animals. Death has occurred in animals as a result of secondary

bacterial infection. This species evidently has extended its range into Indiana from the north.

Another case of dermal myiasis occurred in a very old and feeble dog. Larvae of *Phaenicia sericata* (Mg.) and *Phormia regina* (Mg.) were recovered from the frontal head region on August 26. Identification of these flies was confirmed in the adult stage.

It is doubtful if primary screwworm occurs in this area; however, a survey is planned in 1972 which will solicit the cooperation of veterinarians in Indiana for early reporting of all myiasis cases, so that accurate species determinations can be made, and follow-up studies conducted at sites where actual infestations occur.

Preliminary Investigation of the Effects of Three Hormonomimetic Compounds on Larvae of *Culex pipiens pipiens* (L.) (Northern House Mosquito). STEVE BETRAS and R. E. SIVERLY, Department of Physiology and Health Science, Ball State University, Muncie, Indiana 47306.—The effects and lethal dosages of three hormonomimetic agents were investigated during the summer of 1971. Tests with Stauffer (R-20458), Monsanto (Mon-0585), and Zoecon (ZR-0515) were conducted, using late instar larvae of *Culex pipiens pipiens* obtained from waste lagoons in Delaware and Henry Counties. All three compounds were juvenile type hormones.

Larvae were exposed to the chemicals by dissolving the compounds in acetone and mixing these solutions with lagoon water in which larvae were collected. Each sample consisted of 20 larvae in 100 milliliters of filtered lagoon water. Control samples were set up with filtered lagoon water alone. From three to five levels of concentrations were used on each trial, with from three to five replicates for each level of concentration.

Effective dosages were much higher than the manufacturer's recommendations for each compound. This was believed due to rapid biodegradation of the chemicals in lagoon water.

R-20458 and Mon-0585 produced a total kill during a 48-hour exposure at 40 and 30.5 parts per million, respectively. ZR-0515 produced complete kill at 15.5 parts per million with a 24-hour exposure. Most of the tests were difficult to evaluate because of the high mortality in the controls. The Zoecon product appeared more active, but less stable than either the Stauffer or Monsanto compounds. Further laboratory investigations should be conducted before actual field testing of these compounds is attempted.

A Preliminary Study of Autogeny and Host Preference of *Culex pipiens pipiens* L. (Northern House Mosquito) in East-Central Indiana. DONALD A. SHROYER and R. E. SIVERLY, Department of Physiology and Health Science, Ball State University, Muncie, Indiana 47306.—Several colonies of *Culex pipiens pipiens* L. were established from immature stages collected at two food processing waste lagoons to evaluate the effects of photoperiod on the expression of autogeny and on host preference.

Three cages were maintained under a 15L 9D photoperiod, at 80° Fahrenheit $\pm$ 2°. One cage was provided no blood host and produced no egg rafts after several weeks. All dissected females from this cage were nongravid.

A second cage was provided only avian hosts, while a third was offered mammals. Although *Culex pipiens pipiens* is regarded as primarily an avian feeder, samples from both cages contained equivalent proportions of engorged females. However, females fed on birds produced more eggs than those fed on mammals.

Two colonies were maintained under a 13L 11D photoperiod. One cage was provided a bird host and the other a mammal. There was no evidence to support the shorter day length as conducive to mammal feeding, since samples from both cages contained equivalent proportions of engorged females.

Further studies to clarify the effects of photoperiod on host preference and autogeny will be conducted by: 1) observations of laboratory colonies under simulated natural light conditions but constant temperatures, 2) observations of laboratory colonies under simulated hibernating conditions, and 3) studies of natural overwintering populations in the field.

Genetic Studies on Melanic Forms of *Tropisternus collaris* (Fabricius) from North and South America. FRANK N. YOUNG, Department of Zoology, Indiana University, Bloomington 47401.—Laboratory studies of the results of crossing melanic *Tropisternus collaris* from South America with the melanic subspecies from Florida and South Carolina indicate higher levels of interfertility than shown in crosses with the lightly pigmented North American forms. Rearing of larvae from wild caught females from South Carolina show that interbreedings of light and melanic forms occur in nature. Further evidence for the discreteness of the genes producing the melanic condition was presented.

The Planipennia (Neuroptera) in Indiana. H. RANDOLPH LAWSON, Department of Entomology, Purdue University, Lafayette, Indiana 47907.—Representatives of nine families (Coniopterygidae, Mantispidae, Berothidae, Hemerobiidae, Polysteochotidae, Sisyridae, Chrysopidae, Ascalaphidae, and Myrmeleontidae) were recorded in this study. Representatives of the eighteen species previously recorded in literature as occurring in Indiana were collected during the summer of 1969. Twenty species were reported as being new records from Indiana. Due to inadequate taxonomic work on the species of the family Coniopterygidae, specimens of this family were identified only to genus. Five genera of coniopterygids were recorded.

The possible occurrence of 17 more species which have been recorded from adjacent areas is reported. Included in these possible species is *Nallochius americanus* (McLachlan) which represents a 10th family, Dilaridae. Therefore, a total of 55 species is included in the keys constructed for adult identification.

Important range extensions include *Micromus variolosus* Hagen, a western species of Hemerobiidae, and *Brachynemurus longicaudus* (Burmeister), an eastern, south-eastern coastal species of Myrmeleonidae.

The only recorded species of the family Polysteochotidae in the eastern U. S., *Polysteochotes punctatus* (Fabricius), appears to have had a drastic population decline since the early 1900's. Although collected quite often in the Chicago area around the turn of the century, no specimens have been seen that were collected after 1940.

OTHER PAPER READ

Fruit Insect Research Programs of the U. S. Department of Agriculture.
M. L. CLEVELAND, USDA, ARA, Beltsville, Maryland (by invitation).

Indiana State Records and Notes on Some Rare Butterflies and Skippers in the State

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Abstract

As the result of collecting butterflies in the state from 1964 to 1971, six species, *Thymelicus lineola* (Ochsenheimer), *Polites mystic* (Scudder), *Autochton cellus* (Boisduval & LeConte), *Erynnis baptisiae* (Forbes), *Euristrymon ontario* (Edwards), and *Polygonia satyrus* (Edwards), have been added to the butterfly fauna of Indiana. In addition to the new state records, the following rare species were collected: *Euphyes dukes* (Lindsey), *Satyrrium caryaevorus* (McDunnough), and *Pieris napi oleracea*.

Family Hesperiiidae

Thymelicus lineola (Ochsenheimer)—European Skipper

I collected one male June 22 and one female July 1, 1967, in a grassy pasture, North Manchester, Wabash County. In recent years I have collected *lineola* in LaGrange, Elkhart, Kosciusko, and Wabash Counties (3). Pairs *in copula* have been taken in June, suggesting that this species has become well established in Indiana.

Polites mystic (Scudder)—Long Dash

I collected the first *Polites mystic* in the state on June 6, 1969, Mongo Bog, Mongo, LaGrange County, only seven miles from the Michigan border (6). It is now fairly common in Wabash and Kosciusko Counties. June 10, 1970, I found a colony of this species in a bog by Lake Waubee, Milford, where *Iris versicolor* was its favorite food flower.

Autochton cellus (Boisduval & LeConte)—Golden Banded Skipper

Single specimen was collected by my son, James D. Shull, June 7, 1970, in Brown County State Park, near Nashville, Indiana (7).

Erynnis baptisiae (Forbes)—Wild Indigo Dusky Wing

August 27, 1970, I collected a female near its food plant (*Baptisia tinctoria*) in a field near North Manchester but in Kosciusko County (7).

Euphyes dukes (Lindsey)—Dukes' Skipper

Mr. Homer F. Price, Payne, Ohio, collected one male July 24, 1962, in Steuben County (2). I collected one more June 27, 1970, North Manchester, Wabash County (7).

Family Lycaenidae

Satyrrium caryaevorus (McDunnough)—Hickory Hairstreak

I collected a pair June 22, 1968, on a hickory tree leaf in Kosciusko County (5). *Caryaevorus* is uncommon in Wabash and Kosciusko Counties in June and July.

***Euristrymon ontario* (Edwards)—Northern Hairstreak**

Collected the first specimen July 12, 1967, on dogbane flowers, North Manchester, Wabash County (4). Each year a few have been collected in Wabash County from late June to about mid-July.

Family Nymphalidae***Polygonia satyrus* (Edwards)—Satyr Angle Wing**

June 25, 1969, I collected this essentially western species in Kosciusko County, Indiana (6). It is uncommon, rare, or absent from late May through July in Wabash and Kosciusko Counties.

Family Pieridae***Pieris napi oleracea* Harris—Mustard White**

Blatchley (1891) reported this species from Kosciusko County, in the summer of 1890 (1). Old records are unreliable as *napi* was often confused with *virginiensis*.

July 12, 1971, John Campbell, a high school student in my collecting party, collected one in Mongo, LaGrange County. On August 24 John Campbell and I returned to Mongo, where we collected 23 more *napi*. It should be looked for elsewhere in the state.

No doubt other rare Indiana species could be added to this report, but Mr. S. Sidney Badger, Jr., of Woodland Hills, California, and I have just completed a comprehensive annotated list of the butterflies of Indiana, 1971, therefore I have limited this paper primarily to a listing of my own state records.

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Aggregations of *Chalybion californicum* (Saussure) (Hymenoptera: Sphecidae) near Centerville, Wayne County, Indiana

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Abstract

Observations over a 5-year period of aggregations of *Chalybion californicum* (Saussure) near Centerville, Indiana, indicate a preference by this wasp for a western exposure on wooden buildings. The wasps were found at the nighttime roost sites, in varying numbers from about June 1 to September 30. A peak in numbers was reached between June 14 and June 28, with a second, but much lower peak coming from 6 to 22 days later. The highest number counted was 260 on June 26, 1971.

Introduction

Clusters of animals are of interest to biologists for several reasons. Sleeping aggregations permit easy population censusing. Aggregation is of interest as a precursor of social development, of particular significance when occurring in an otherwise solitary species. Finally, there is little understanding of the stimuli which initiate grouping and which hold groups together.

Aggregations of the blue wasp, *Chalybion californicum* (Saussure), were observed over the 5-year period from 1967 through 1971 near Centerville, Wayne County, Indiana. Observations extended from June 1 to October 1.

Over the years, several explanations have been offered for clusters of animals. In 1916, Fabre (5) thought that the cluster of several hundred wasps, *Podalonia hirsuta* (Scopoli), which he found sheltering under a stone might be resting migrants. After studying the sleeping habits of a Florida butterfly, *Heliconius charitonius* L. (= *H. charithonia* L.), Jones (7) argued the pros and cons of group protection. He observed that when one insect was disturbed the whole group was warned, but also found that a predator could gorge on the collected insects if they were unwary or slow to move. He saw no sexual significance in grouping. Evans and Linsley (4), observing bees and wasps of several species, found no clear adaptive value in sleeping aggregations. However, in clusters of the digger wasp, *Steniolia obliqua* (Cresson), which Evans and Gillaspay (3) observed for 5 years, mating behavior was evident. Evans believed that the clustering served a social function leading to mating.

Allee's (1) interest in aggregations is well known. After referring to some accidental animal groupings such as New England corals and the animals on a wharf piling he said, "Other close aggregations occur as a result of the less spectacular trial and error reactions, in which the animals wander here and there, more or less vaguely stimulated by internal physiological states or external conditions, and so come to collect in favorable locations." The effect of one external condition,

temperature, on the choice of an aggregation site was described by El Rayah (2) for 2 tenebrionid beetles, *Adesmia antiqua* Klug and *Pimelia grandis* Fabricius. He found that *A. antiqua* aggregated in a zone of 34-38°C and *P. grandis* gathered in a zone of 28-34°C. *Chalybion zimmermanni* Dahlbom which P. Rau (10) saw in Mexico may have been responding to temperature. Rau found about 20 of these blue wasps clustered on a piece of rope in a shed where a 75-watt incandescent lamp burned until midnight.

Hamilton *et al.* (6), after studying an aggregation of starlings (*Sturnus vulgaris* L.), devised a model of a refuge with a core at the center representing the nighttime roost, and two dispersal areas around it. They believed that the expenditure of time and energy required to fly from the core to the outer range was balanced by the lack of competition in that area. Applying this model to *C. californicum*, the nighttime roost is the core, the nest area lies in the inner dispersal area, and the feeding area is the outermost range.

An aggregation pheromone was isolated from the southern pine beetle, *Dendroctonus frontalis* Zimmermann, by Payne (8). He found that the chemical was emitted in the frass by female beetles and was attractive to both males and females of the same species.

Variations in the numbers of *Chalybion californicum* at nighttime roosts were reported by Rau and Rau (11) and by P. Rau (9) from Missouri and Kansas. For example, 30 wasps were found on the underside of an overhanging rock near the Meramec River in the vicinity of St. Louis, and 100 or more wasps were found in an open cow shed in the middle of July at Lake View, Kansas (11). Near Wickes, Missouri, a group of more than a thousand were in the eaves of an abandoned house. Another group slept in a niche of a pillar and a third group entered a hollow tile on top of a brick chimney (9).

In New Jersey, Weiss (12) found a group of *C. californicum* clustered at night on the same inside corner of a cloth awning for 5 summers. These were on the southwest side of a dwelling (personal communication). Leland Chandler (personal communication) reported that a group of *C. californicum* roosted on the west-facing side of a shed near Otterbein, Indiana.

It seems possible that the initial choice of a roost or refuge by *C. californicum* may be based on temperature. The continued use of a roost then may be reinforced by a pheromone or some other odor-producing material.

Results of Observations

The wasps started gathering at the main roost site in east-central Indiana about June 1 and several could be found there until the middle of September. Roosting began about 2 hours before sunset, and the last wasps had settled by about an hour after sunset. During the period of settling they would fly off if an observer approached within 3 feet. Counts were made after dark when the wasps were not disturbed by

the beam of a flashlight. In the morning the wasps were often still in place until 2 or 3 hours after sunrise. They were active earlier on warm, sunny days than on cool, cloudy days.

At one favorite roost site, an old privy, a Fahrenheit thermometer was fastened close to where the wasps spent the night. Temperature was recorded each time a count was made. When temperature at the roost site was compared with that on the north side of a nearby building, the roost site was warmer by 4°F.

Over the 5-year period several roost sites were used. The main site favored by most of the wasps changed from the west side of the privy to the west side of a tool shed (Fig. 1). The tool shed was about 7 feet east of the privy and the roost site under the eaves was about 4 feet higher than the former site. Sunlight reached the new site for several minutes longer each day.

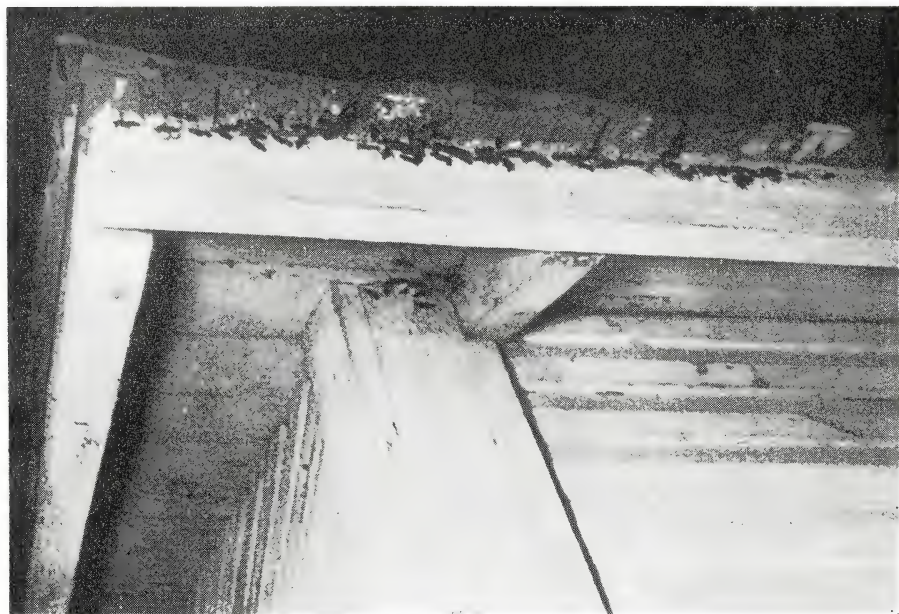


FIGURE 1. *Aggregation of Chalybion californicum* (Saussure) on west side of tool shed near Centerville, Indiana, on night of June 15, 1970.

Early in June 1971 a few wasps were found on the north side of the tool shed just under the peak of the roof, and a few were on the southeast corner of the shed. As the summer progressed wasps disappeared from these sites and more accumulated on the west side. A few also appeared around a seldom-used light socket on a porch ceiling and also under a loose shingle on the south side of a building about 5 feet north of the tool shed. The shingle gave some protection from the weather and, at the end of the summer, wasps were found here in small

numbers after they had disappeared from the main site. For example, the last wasps were seen on the west side of the tool shed on September 9, 1971, but one was found under the shingle as late as October 1, 1971.

Two peaks in numbers of wasps occurred in each of the 5 years. Except in 1969, the first appeared in June and the second in July. In 1969, both peaks were in June. During the study, time between peaks varied from 6 to 22 days (Table 1). During 3 summers, counts were made of female *C. californicum* working on nests in the tool shed and other buildings, but there was no correlation of these counts with counts of the roosting wasps. The number of nesting wasps was always low, about 5 to 10.

TABLE 1. *Numbers of roosting Chalybion californicum* (Saussure) near Centerville, Indiana, 1967-1971.

Year	First peak	Date	Second peak	Date	Days between peaks
1967	100	June 28	60	July 4	6
1968	50	June 21	30	July 8	17
1969	16	June 7, 8, 9	13	June 25	16
1970	132	June 14	54	July 2	18
1971	260	June 26	45	July 18	22

No predation on the wasps by birds was observed at the roost sites over the 5-year period, although birds which are known to eat wasps were in the vicinity. The extent of predation away from the roost site was not investigated.

Aggregations were made up of both males and females. No mating behavior was observed at the roost sites.

Small deposits of fecal matter were found at the roost sites. These were not tested for presence of a pheromone because techniques for the analysis of such small amounts of material were not accessible.

Conclusions

1) It is believed that the warmer temperature at the main roost sites on the west sides of 2 wooden buildings was the initial stimulus for choice of sites.

2) Peaks in numbers of wasps at the roost sites occurred from 7 to 28 June, over a 5-year period.

3) Peak numbers of wasps varied from 16 to 260. Second but lower peaks varied from 13 to 16 and followed from 6 to 22 days later. Some of the second peaks were higher than some of the first peaks of other years.

4) Neither mating behavior nor predation by birds was observed at the roost sites.

Acknowledgements

The author is grateful to Jay A. Myers, Lee J. Reynolds, Sarah C. Strawn and Carl H. Luckhardt for their assistance during the observations.

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Observations of *Halictus confusus* Smith (Hymenoptera:
Halictidae) on Woodland and Field Flowers¹

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Abstract

The native bee (*Halictus confusus*) has been recorded from at least 165 host plant species. Observations were made of a local nest population for 4 years. These indicated that the flowers visited by bees during the warm months of the year reflected availability and, possibly, selectivity. Events of 1964 were typical and are reported.

Spring beauty (*Claytonia virginica* L.), toothwort (*Dentaria laciniata* Muhl.), dandelion (*Taraxacum officinale* L.), and winter cress (*Barbarea vulgaris* R. Br.) were visited most frequently during April and May. Nectar and pollen were obtained principally from white clover (*Trifolium repens* L.) and red clover (*T. pratense* (Mill.) Schreb.) during the summer period of nest expansion.

The native bee, *Halictus confusus*, is a polylectic species with a wide range of floral hosts throughout the growing season in the eastern United States (3). While studying the ecological life history of this species at the Purdue University Entomological Research Area near West Lafayette, Indiana, in 1964, observations were made of the interrelationship of a nesting population of *H. confusus* and plants having conspicuous flowers within 200 yards of the nesting site from April to October.

Although *H. confusus* has been recorded on at least 165 species of plants (1, 2, 3), this extensive floral record does not reflect the utilization of regional flora by a local nesting population. In the results reported here, only 22 plant species were frequented in the forage area by the bee colony investigated, and only 6 species were considered important to colony nutrition (Table 1).

During April, the newly-emerged queen bees showed a marked preference for spring beauty (*Claytonia virginica* L.) in the woodland areas adjacent to the colony site on a field hilltop. At this time, the trees were still without foliage and the forage area of the woodlands was well lighted in favorable weather. Most of the floral visits to spring beauty were for nectar to be used in personal nutrition, although several observations of pollen feeding and one sighting of pollen collection on the scopae were recorded on this plant. Pollen collection was indicative

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³Acknowledgment: Especial appreciation is acknowledged for the advice and guidance of Dr. Leland Chandler during the course of this study, which is a phase of the research for a doctoral thesis.

of early nest provisioning for the next brood generation. Toothwort (*Dentaria laciniata* Muhl.), which bloomed at the same time and in the same areas, was also visited by the females but to a lesser extent.

TABLE 1. *Plants visited by Halictus confusus Smith in the Purdue University Entomological Research Area, 1964.*

Host plant	Range of observation dates	No. days bees observed	Plant product collected ¹
Spring beauty ²	April 8-26	3	N
Toothwort ²	April 23-26	2	N
Dandelion ²	April 23-May 20	6	P
Redbud	May 3	1	N
Winter cress ²	May 4-18	8	P
Shepherd's purse	May 6	1	N
Mouse-ear chickweed	May 18	1	N
Blackberry	May 22	1	N
White clover ²	May 22-July 30	11	P
Yarrow	June 5	1	N
Hop clover	June 9	1	N
Red clover ²	June 10-July 29	15	P
White sweet clover	June 19	1	N
Crown vetch	June 27	1	N
Wild rose	June 29	1	N
Yellow sweet clover	July 3	1	N
Wild carrot	July 13-16	2	N
Daisy fleabane	July 16	1	N
Horse nettle	July 19	1	N
Japanese lespedeza ³	September 5	1	N
White heath aster ³	September 18	1	N

¹Symbol (P) denotes pollen and nectar being collected, while (N) represents nectar only.

²Multiple observations on date of observations.

³Only male(s) observed on host plant.

As these woodland flowers waned in late April, the bees shifted suddenly to dandelion (*Taraxacum officinale* (L.) Weber) as this flower attained a conspicuous bloom in the fields near the nesting site during the last week of April and the first few days of May. The visits to dandelion blossoms were primarily for personal nutrition. About the first week of May, winter cress (*Barbarea vulgaris* R. Br.) began to bloom in large conspicuous stands in nearby orchards and fields. As it became common, *H. confusus* females deserted the dandelions and for the next several weeks were found on the blossoms of this plant during favorable weather. Coincidentally, provisioning of brood cells in the nests became commonplace as this floral species began to bloom. The pollen and nectar collected from winter cress probably provided

more than 95% of the material from which the pollen balls of the first brood were fashioned by the overwintered females. By the end of the third week in May, the last utilization of this plant was witnessed as it entered a fruiting stage.

During late May, flowers in the nearby fields and orchards were scarce and the bees were observed spending more time than usual in the field, presumably searching for the few winter cress blossoms still left or looking for alternate sources of nutrients. At this time, the longest flights of 1964 took place with 10 flights for pollen, taking from 60 to 138 minutes, whereas the seasonal average was about 30 minutes. At this time, single observations were recorded for mouse-ear chickweed (*Cerastium vulgatum* L.) and blackberry (*Rubus* sp.), suggesting that a search for a preferred floral host was in progress.

At the conclusion of this period of flower scarcity, the females became inactive and the nests became sealed as the adults remained in the nests with their developing broods. This period extended from May 26 to June 6, 1964. As the first brood-worker females emerged, conspicuous flowers were in bloom in the forage areas, such as yarrow (*Achillea millefolium* L.), daisy fleabane (*Erigeron strigosus* Muhl.), and white daisy (*Chrysanthemum leucanthemum* L.). Although a few visits were recorded to these composites (white daisy visited by *H. confusus* on June 5, 1965), the summer workers favored white clover (*Trifolium repens* L.) and red clover (*T. pratense* (Mill.) Schreb.) as the major sources for nectar and pollen during the June and July nest expansion and provisioning period. During this time, *Halictus ligatus* Say, a related species coexisted with *H. confusus* by nesting in the same nesting site. However, interspecific competition was apparently avoided in part by preference of floral hosts since *H. ligatus* was common on the flowers of yarrow, daisy fleabane, and white daisy and uncommon on white clover and red clover, just the opposite of *H. confusus*.

Halictus confusus adults were observed 1 to 3 times only on redbud (*Cercis canadensis* L.), shepherd's purse (*Capsella bursa-pastoris* (L.) Medic.), hop clover (*T. agarium* L.), white sweet clover (*Melilotus alba* Des.), wild rose (*Rosa* sp.), yellow sweet clover (*M. officianalis* (L.) Lam.), horse nettle (*Solanum carolinense* L.), wild carrot (*Daucus carota* L.) in the order first observed in the field.

In late summer and fall as clovers became scarce, the newly-emerged males and females fed on the available flowers, such as Japanese clover (*Lespedeza lineata* (Thumb.) H. and A.) and white heath aster (*Aster ericoides villosus* (Mich.) Voss). Since these females were destined to be the overwintering brood, they did not construct cells and collect pollen provisions.

The only other nutrient observed to be collected by *H. confusus* was perspiration, presumably for the salt content. Although Stevens (4) reported this species to be a common "sweat bee" in North Dakota, the behavior of "sweat bees" in feeding on the perspiration of one's bare skin was not observed in *H. confusus*, nor could it be induced. In the nesting site, *Dialictus imitatus* (Smith) and a larger species,

probably *D. zephyrus* (Sm.), were common "sweat bees" on hot and humid days but were not joined by *H. confusus* individuals. The manner in which this species fed upon perspiration was to land on artificial objects that had been handled such as stakes, paper bags, etc., and feed upon the perspiration residue. There was one record of a female *H. confusus* hovering about the eyes of the observer for several minutes in the manner of an eye gnat.

Some species of flowers seemed to be visited for nectar only. In central Indiana, five species of milkweed were observed to have male and female bee visitors for nectar only. Whorled milkweed (*Asclepias verticillata*) was quite attractive to *H. confusus* during midsummer in the general Lafayette, Indiana, area. Both sexes transported pollinia in interblossom transfer, but these were never found in pollen balls within a nest.

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GEOGRAPHY AND GEOLOGY

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Abstracts

Glacial Geology of St. Joseph County, Indiana (Map, 1:62,500). REV. MICHAEL J. MURPHY, C.S.C., and JOHN P. SZABO, Department of Geology, University of Notre Dame, Notre Dame, Indiana 46556.—Northern Indiana was rejuvenated in the Pliocene producing a gently rolling landscape with moderate relief. The La Porte Valley and Elkhart Valley, two buried valleys, reflect this. Two of three ice lobes which penetrated Indiana produced the presently visible glacial features during the Wisconsin's Cary substage. Some features' depositional histories have multiple explanations while those of others are more evident. One interpretation of the St. Joseph River's present course involves piracy of the headwaters of the ancient Kankakee River. The pebble lithologies of various deposits show the underlying bedrock type; there are differences between outwash and till pebble analyses. Some percentages may possibly serve as indicators in distinguishing a lobe's deposits in this area.

Age and Correlation of Middle Devonian Strata of Jasper County, Indiana. R. WILLIAM ORR and WILLIAM D. REBUCK, Department of Geography and Geology, Ball State University, Muncie, Indiana 47306.—Outcrops of Middle Devonian strata in south-central Jasper County, near Rensselaer, were examined and collected for conodonts as part of a continuing study of conodont biostratigraphy of Middle Devonian rocks of Indiana. Jasper County exposures consist of mostly fine-to-medium grained, dark gray, vuggy dolomite, which lithology is also exhibited by correlative subsurface strata to the south in White, Tippecanoe, and Warren Counties. Conodont faunas of the studied outcrops are dominated by specimens of the important platform genera *Icriodus* and *Polygnathus* and contain as late Middle Devonian zonal indicators *I. latericrescens latericrescens* and *P. varcus s. l.* Middle Devonian strata of Jasper County represent a southwestern dolomitic facies of the Traverse Formation of the Michigan Basin and lie within the *P. varcus* Zone high in the Middle Devonian. These beds correlate with the upper (sub-Squaw Bay) part of the Traverse Group of Michigan, the upper part of the Traverse Formation of northern Indiana, and the Beechwood Member of the North Vernon Limestone of southern Indiana and the Illinois Basin.

Structural Control of Cavern Development in Northwestern Washington County, Indiana. RICHARD L. POWELL, Department of Geosciences, Purdue University, Lafayette, Indiana 47907.—Most caverns in the outcrops area of limestones of Mississippian age in south-central Indiana consist of passages that trend down the local dip of the bedrock. Generally, the regional structure is reflected by numerous caverns or subterranean drainage routes which divert surface karst drainage westward or southwestward to outlets or springs along entrenched surface streams. The gradient of the subterranean passages is generally compatible with the local rate of dip of the bedrock, sometimes dissecting progressively lower strata if the cave stream gradient exceeds the rate of dip. A few cavern passages parallel the strike of the strata, usually owing to a steeper hydraulic gradient to a surface outlet. Examples of subterranean drainage in an updip direction are rare, if not nonexistent, in Indiana.

Several caverns have been surveyed in the drainage basin of Clifty Creek, in northwestern Washington County, Indiana, which exhibit subterranean drainage contrary to the southwestward regional trend. These caverns have commonly developed eastward in a down-dip direction on the east flank of the southernmost nose of the Leesville Anticline. They are tributary to Clifty Creek, a partially strike-oriented, deeply entrenched stream. River (Wet Clifty) Cave is mostly a strike-oriented, northward flowing cavern, a subterranean headward extension of Clift Creek. This example of cavern development in adjustment to local structure is proof of the importance of structural control to the initiation and development of subterranean drainage.

To Harmonize a County—A Proposed Integrated Study of Vigo County, Indiana. JOHN H. CLEVELAND, Department of Geography-Geology, Indiana State University, Terre Haute, Indiana 47809.—The finite nature of a region's natural resources, combined with ever increasing demands upon them, produces a conflict between man and his environment that may lead to extinction, or at best, radical change for both. Challenged by the writings of Indiana "geocologists" Guernsey, Wayne, and Patton, a comprehensive pilot study of Vigo County is underway to form the orderly framework of information necessary for viable courses of action now to avert future tragedy. The project will: 1) compile and catalogue the extensive data and sources represented by federal, state and local governmental agencies, educational and research institutions, and business enterprises; 2) outline and undertake research projects of both short and long term duration to fill in the information gaps; 3) develop "dynamic equilibrium equations" for the local ecosystem; and 4) disseminate this information to the public and to the decision makers. As an illustration, coal resources are examined.

It is believed that this continuing study will aid in justifying our existence, enhance teaching and research capabilities, and of most importance, aid in rational progress towards a dynamic equilibrium with his environment of man's choosing, permitting a quality survival of both for a significant fraction of geologic time.

Geography's Inherent Role in Environment Control. ALFRED H. MEYER, Department of Geography, Valparaiso University, Valparaiso, Indiana 46383.—Regional populations and their optimally related natural and human resource patterns considered in their total environmental perspectives—social, economic, political and spiritual—may be said to be the basic geographic principle of evaluation of human behavior and life's motivations.

Ethnic and economic concerns of man are necessarily space-related and resource-oriented, whatever the stage of cultural development may be. Hence, the study of human ecology of the environment in sequent occupance form has been traditionally considered a comprehensive challenge to the geographer.

Despite historic antecedents and present-day research and teaching concerned with the basic qualities of the environment, the field of geography today occupies a comparably inferior professional position in the area of Environment Management.

This paper explored the reasons for this condition and suggested some remedial measures establishing professional and public recognition of geography as a distinctly environmental behavior science.

Factors Associated with the Decline of Central Place Functions On the Rural Poor of Monroe County, Indiana. STEVEN K. PONTIUS, Geography Department, University of Minnesota, Minneapolis, Minnesota 55455.—Between 1910 and 1970 the number of small towns and villages in Monroe County, Indiana, providing the essential retail and service activities has declined from 31 to 2. The result of this decline has spatially isolated many rural residents who previously were within 2 miles of such a village anywhere in the county. To those individuals who have transportation this is of little or no concern.

However, to over 100 of the 294 low-income households interviewed, which represents a 20% sample of all low-income rural residents in Monroe county in 1965, the ramifications are great. They represent people who have no means of transportation. Characteristically these people are 65 or older, have lower incomes, fewer jobs, poorer housing conditions, and who are spatially more isolated from the remaining central places than the low-income people interviewed who had transportation.

The consequences associated with their isolation, due to their lack of transportation and their household location in relation to the remaining central places are: the inability to supplement their income, and thus the inability to maintain their homes, buy nutritious food, and acquire preventive medical care. And, saddest of all is that because of this spatial isolation, some of these people give up in despair, stay home, and become more isolated from the world. In reality they exist in a sort of solitary confinement.

The Relationship of Expensive Residential Dwellings to Hilly Terrain. HENRY W. BULLAMORE, Department of Geography, University of Illinois,

Urbana, Illinois 61801.—A field study indicated no statistically significant relationship between expensive residential dwellings and hilly terrain in the City of Valparaiso, Indiana. The concept of "hilliness" was quantified through the use of the local relief within designated study sectors. The mean true value according to the Assessor of randomly selected homes within each sector was used to quantify "expensiveness". Analysis using Pearson's Correlation Coefficient r , the Chi Square test, and other tests, all failed to substantiate the hypothesis that expensive homes would be found in hilly areas.

Fulmendosa River: A Multipurpose Irrigation Project in Sardinia. F. P. KALLAY, Geography Department, Valparaiso University, Valparaiso, Indiana 46383.—One of the great engineering triumphs of modern man has been his ability to transform acres of barren land into arable areas of large scale productivity. Such a transformation is occurring in the southwest portion of Sardinia, known as the Campidano, an area which is already an intensively cultivated portion of the island, but possessive of much greater agricultural potential. This potential will be realized in the form of a vast project known as the ENTE AUTONOMO DEL FLUMENDOSA (EAF).

The major purpose of the EAF is the utilization of the hydro-electric basin of the Flumendosa River and its tributaries. In addition to providing water for irrigation purposes, the EAF project will supply water for the capital city of Cagliari and several surrounding communities. Furthermore, it will provide hydroelectric power at an annual average of 80 million kilowatt hours.

The effects of the entire EAF project will not be known in their totality for several more years, but it is safe to say that the effects will be far-reaching for the island of Sardinia and will have numerous benefits on the Italian economy as a whole as well.

Coal Age Tree Stumps in Indiana. D. L. DILCHER, Botany Department, Indiana University, Bloomington 47401.—During the spring of 1971 the Paleobotany class of Indiana University located three casts of Sigillarian trunks apparently standing in place where the trees once grew. These were found exposed in the No. 5 pit at the Hawthorne strip mine of Peabody Coal Company. Several more trunks were located during the summer and one was collected by the Indiana State Natural History Museum before mining was stopped in the area. The nature of the trunks, positions, size and relationships were discussed and a photographic record of them was used to illustrate the talk.

OTHER PAPER READ

A Study of the Floras in the Allegheny Series in Green County Indiana. R. N. PHEIFER and DAVID L. DILCHER, Indiana University.

Earth Dam Problems—and the Geological Site Investigation

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Abstract

There are many small man-made lakes in Indiana and a few large reservoirs with dams over 100 feet in height. The major problems encountered in the construction and operation of these structures are seepage around or through their fills, permeability or weakness of foundation bedrock, unsuitable construction materials, instability of natural slopes, subsidence of dam or pool into caverns or mine voids, and tectonic activity. Faulty construction is not within the scope of this paper. To minimize structural inadequacies or failures caused by these problems, the design engineer must be forewarned. An adequate detailed design investigation must be conducted by a capable geologist. The geologist must review all available data relative to the site, study, test and sample the features of the site, submit samples, test data and his interpretations to the soil mechanics laboratory, and present all this information and the laboratory report to the design engineer.

Introduction

Multi-purpose use of stored surface water has increased greatly in recent years. Problems in dam construction, operation and maintenance have kept pace.

In Indiana most dams for water storage have been constructed with earth. The type of earth material is dictated by the type of dam, the site features and the available material.

Problems are encountered during construction and while the reservoir is in operation. The purpose of the geological dam site investigation is to discover the problems so that they may be offset by adequate design.

Simply expressed, a dam must be strong enough to hold back a given volume of water. The permeability and strength of the dam and its abutments are important factors in the successful operation and maintenance of the reservoir.

Pre-design investigations of dam sites vary in intensity. The larger and more elaborate the structure, the more detailed must be the investigation. The compilation and review of data on dam failures or deficiencies has largely determined the steps taken in current dam site investigations.

The causes of inadequacies in all earth fills reported prior to 1952 have been placed in the following order (5) :

1) Overtopping	30%	5) Slope paving	5%
2) Seepage	25%	6) Miscellaneous	7%
3) Slides	15%	7) Unknown	5%
4) Conduit leakage	13%		

Most of the dams referred to in this study are large structures. The writer believes that most of the problems could have been foreseen had an adequate pre-design been employed.

Overtopping usually occurs when the dam has insufficient height or when the outlet structure is inadequate. This design deficiency usually results from inaccurate hydrologic data. In recent years the science of hydrology has become much more reliable and overtopping is no longer a common event.

Based upon the writer's experience and study of recently constructed earth dams in Indiana, the following order for problems encountered is listed as follows:

- | | |
|----------------------------------|----------------------------|
| 1) Seepage in overburden or fill | 3) Bedrock incompatibility |
| 2) Foundation weakness | 4) Miscellaneous. |

Seepage occurs through permeable fills, abutments and/or foundations. This leakage results in water loss and, frequently, piping of fine soil materials in fill or adjacent natural ground. Poorly graded (well sorted) sands and gravels are common offenders. Abutment or foundation seepage may occur through layers or lenses of sands and gravels in glacial drift or in alluvial sediments. Fills constructed chiefly with coarse-grained earth materials are subject to seepage. Conditions conducive to either of these possibilities must be recognized in the pre-design investigation. Permeable materials are important components of a good fill, but must be used with silts and clays.

Soft and compressible foundations will consolidate during or after construction. Weak soils located at the base of the dam must be found, sampled and tested. Such weak materials must be either treated or removed. Standard penetration tests, conducted during the site investigation, pin-point weak foundations. Undisturbed samples of the weak material, as indicated by standard penetration tests, are collected and submitted to a soil mechanics laboratory for test data. These data determine the proper solution for the design engineer.

Shale, sandstone and limestone are the bedrock possible to encounter during the construction of dams in Indiana.

Shale is generally impermeable and of adequate strength. Slides and creep occur on hillsides where shale is near the surface. Some shales swell when wet and shrink when dry. Propensity to cause slides or to swell or shrink must be noted in the investigation report.

Sandstone is generally strong and relatively impermeable. Some sandstones are weakly cemented and are therefore permeable, weather rapidly and are often weak. When permeability or strength is in question, adequate testing must be done.

Limestone, when massive and not jointed, provides a good foundation and basin for dams. Cavernous, open and jointed limestone presents problems in maintaining water levels in the reservoir and in making secure the embankment and structural works. When limestone is suspected of

being permeable, the bedrock is usually pressure-tested in place. Core samples may be subjected to testing to determine stability.

There are numerous miscellaneous problems that may result in the inadequacy or eventual failure of a reservoir if not discovered in the investigation and allowed for in the design.

Lack of and/or insufficient suitable embankment material may present a serious problem. Sands and gravels, to the exclusion of fine grained soil, or vice versa, are unsatisfactory fill materials.

Haul distance is an important factor in determining the economics of borrow operations. In the construction of the moderate size dams of from 20 to 50 feet of fill height, borrow must be available within 1/4 mile.

Proper placement of fill, proper compaction and satisfactory moisture control are necessary to avoid construction and operation problems (6).

Steep slopes and shallow soils combine to present slope stability problems. During construction, care must be exercised to prevent disturbing slope equilibrium. Unloading the toe of a natural slope may be inviting disaster. Saturation of an otherwise stable slope can also cause a slide. The disaster at the Vaiont reservoir in Italy is an example (3).

Subsurface mining and subsequent subsidence can ruin a dam or its impounded pool. Underground voids resulting from the pumping of oil is believed to be a major contributor to the failure of the Baldwin Hills Reservoir in California (4). Subsidence from coal mining or from oil pumping are not known to have caused damage to dams in Indiana although there is ample reason for concern.

Problems related to tectonic activity have not yet been reported in Indiana. One study of the location of earthquake probabilities indicates that southwestern Indiana is an area of concern (1). There are known and suspected fault areas in the state, many of which appear to be related to the Mt. Carmel fault.

To minimize the hazards related to site problems a pre-design geological investigation of the site is mandatory. This should be planned by and carried out under the direction of a qualified geologist. Minimum requirements for this investigation appear below.

First, all available published or unpublished data relative to the site should be assembled and reviewed. One source of these data is the Indiana Geological Survey.

Second, an adequate subsurface investigation must be carried out in conjunction with a study of all surface features of the site. Field testing, sample collection, and complete and accurate reporting of the site conditions are necessary parts of this requirement (2).

Third, the samples collected must be submitted to a soil mechanics laboratory for testing, analyzing and reporting.

Fourth, the geologists report and the laboratory report must be delivered to and interpreted for the design engineer. An important part of these reports is a recommendation for further study, if necessary.

Fifth, the geologist should make periodic visits to the site after construction has begun to compare conditions actually encountered with those anticipated as a result of the pre-design investigation.

It is my opinion that a well-conceived and an economical site investigation will uncover virtually all adverse site conditions likely to result in dam inadequacies and possible failures.

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Glacial Stratigraphy of the Fort Wayne Area and the Draining of Glacial Lake Maumee¹

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Abstract

Two major glacial till sequences are present in the Fort Wayne area. The upper sequence consists of the clayey tills of the Lagro Formation and the lower consists of extremely hard loam tills that are correlated with the Trafalgar Formation. Both are Wisconsinan in age.

The morphology and development of the Fort Wayne Outlet, the Six Mile Creek channel, and the Wabash-Erie Channel were controlled by a combination of bedrock topography, early glacial discharge routes, the lithology and thickness of glacial materials, and the most recent ice-controlled drainage.

The morphology of the lake floor and the morphology and postglacial stratigraphy of the outlets of glacial Lake Maumee suggest that all three of the classic lake levels could have discharged at Fort Wayne and that the classical 800-foot, 760-foot, and 780-foot sequential order is without foundation. The lower hard tills could have slowed temporarily the downcutting of the Fort Wayne Outlet and stabilized the intermediate lake level.

Introduction

An ongoing study of the environmental geology of the Fort Wayne area, Allen County, Indiana, has required that we develop much more detailed knowledge of the glacial stratigraphy than has been known heretofore. Therefore, we have accumulated a considerable amount of data in the form of water well records, engineering boring logs, shallow refraction seismic records, measured sections of natural and artificial exposures, and logs of our own power augering. Our synthesis of these data is just beginning, but we wish here to outline some of the new information by giving a basic stratigraphic framework for the area and a more detailed description of the outlets of glacial Lake Maumee and then to point out some of the ramifications regarding accepted concepts of glacial and glacial lacustrine history.

Past Work

Until the present study, the most recent geologic work in the Fort Wayne area was done as a part of the Indiana Geological Survey's regional geologic mapping program. The surficial geology shown on the "Geologic Map of the 1° x 2° Fort Wayne Quadrangle . . ." (G. H. Johnson and S. J. Keller, in preparation) does not differ significantly from that mapped by earlier workers (Fig. 1).

The first comprehensive work in this area, the "Report upon the Geology of Allen County," by C. R. Dyer, M.D. (3), has gone virtually unchallenged to this day. And even it, in part, summarized work by

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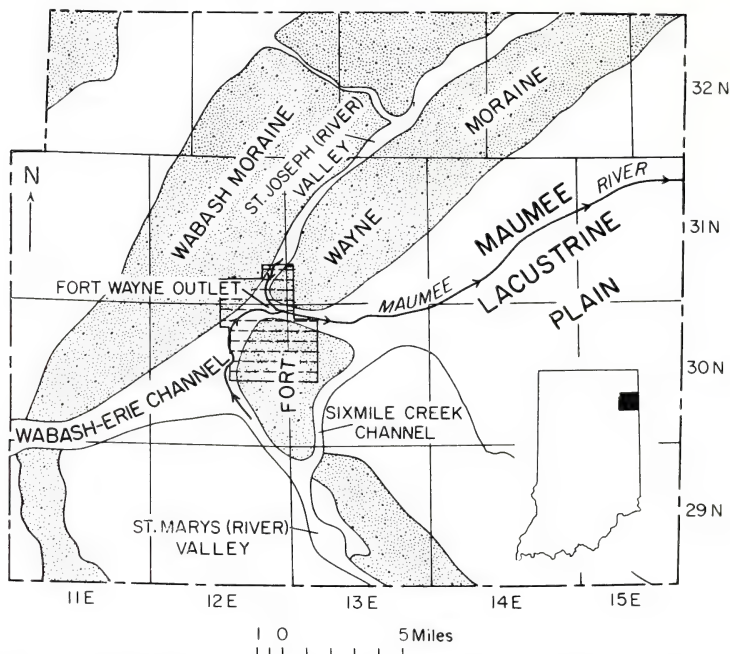


FIGURE 1. Primary geologic features, Allen County, Indiana. Unlabeled areas are till plain. City of Fort Wayne is outlined. Generalized from Johnson and Keller (in preparation).

N. H. Winchell and G. K. Gilbert on "the moraines of the Maumee Valley [that] were among the first to be recognized upon this continent" (4). A report on "The Roads and Road Materials of Allen County," by J. A. Price (10), included a colored 1:250,000-scale surficial geologic map, which is still a valuable tool. The synthesis of this geology into the regional interpretation of glacial history was done by Leverett (8) and Leverett and Taylor (9). There are very few areas in the Midwest whose glacial geology was better known before 1915 than was the glacial geology of this area. And as is true for so many other areas, virtually no morphologic or stratigraphic work has been done since the time of Leverett and Taylor.

Bedrock Topography and Preglacial Drainage

Situated on the northeast flank of the Cincinnati Arch, just within the Michigan Basin, the bedrock surface in Allen County is one of physiographic transition. The dolomite, limestone, and shale bedrock of Silurian to Mississippian age dips very slightly to the north and east. To the north the average bedrock elevation becomes less where valleys are cut into the softer shales. A carbonate rock plateau occupies the south half of the county, and a persistent escarpment separates it from the lowlands in the northern two tiers of townships (Fig. 2) (2). Glaciers from the Erie basin entered Indiana by riding obliquely up the slope of this escarpment.

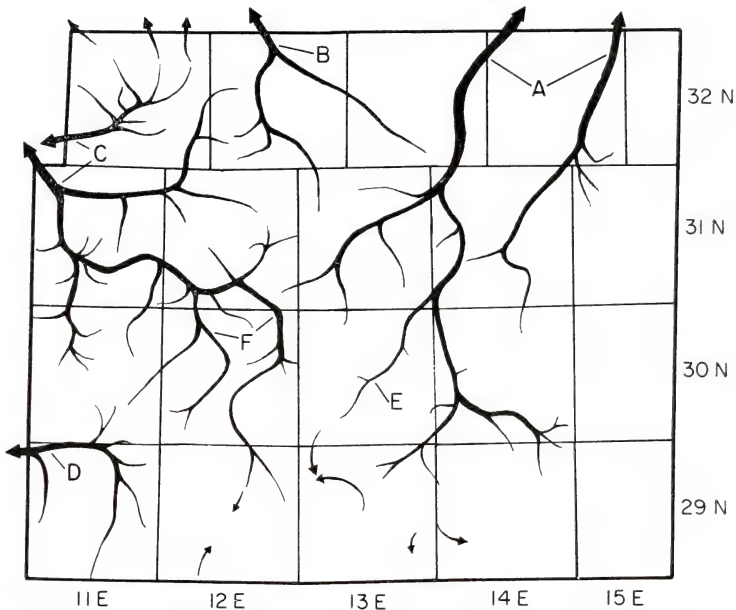


FIGURE 2. Generalized drainage pattern on the bedrock surface, Allen County, Indiana. Generalized from large-scale bedrock topographic map (M. C. Moore, unpublished) compiled from more than 1,000 well, boring, and seismic records.

All bedrock valleys in Allen County are tributary to the Teays Valley, about 50 miles to the southwest (2). The headwaters of the Valley F-C (Fig. 2), which are much steeper than those of Valley E-A, perhaps were deepened by proglacial waters of one of the pre-Wisconsinan ice advances that undoubtedly covered the area.

Glacial Stratigraphy

Two major drift sequences are present in the Fort Wayne area (Fig. 3). A dense sand is commonly present at the base of the lower sequence, and its distribution appears to be partly controlled by topography on the bedrock surface, the thicker deposits commonly occupying lows. The lower drift sequence is composed of loam-textured glacial tills (C and D, Fig. 3), having in places abundant interstratified sand, silt, and flowtill-type deposits, particularly near the top of the unit, and a persistent sand at about mid-depth. The tills typically contain 35 to 45% sand and 15 to 20% clay (grain-size analysis by American Association of State Highway Officials Designation T-88; Wentworth size grades). They are overconsolidated and are hard in the engineering sense of the word, having unconfined compressive strengths far in excess of 4 tons/ft². Moisture contents are typically well below 10% and, correspondingly, dry densities commonly exceed 140 lb/ft³. The top of the lower till sequence rises from an elevation of about 735 feet in the axis of the Maumee Lake basin in easternmost Allen County to an elevation of more than 800 feet beneath the moraines in western Allen County. Between the upper and

The upper drift sequence is the series of clayey tills of the Erie Lobe (Fig. 3, A and B) that make up the concentric moraines of northeastern Indiana and northern Ohio. The upper tills are silty clay-loam to clay-loam textured, containing roughly 10% sand and 40 to 50% clay. They are stiff to very stiff in the engineering sense, having unconfined compressive strengths of 2 to 4 tons/ft². Their moisture content is typically between 15 and 20% with dry densities ranging from 115 to 125 lb/ft³.

A. M. Gooding ("Characteristics of late Wisconsinan tills in eastern Indiana," in preparation) gives evidence for the glacial retreat and subsequent readvance of ice to the Wabash Moraine on the basis of a marked increase in clay content of surface tills within the Wabash Moraine. Although we have not as yet confirmed this in vertical section, numerous engineering boring and water well records and some measured sections indicate a distinct break marked by sand, gravel, or laminated clays within the upper till, which may represent the interval described by Gooding.

The sequential relationship is quite straightforward in most places, but in the Fort Wayne Outlet and the Wabash-Erie Channel areas, as discussed below, the lower drift sequence generally becomes much more complex and the basal sand, the intermediate sand of the lower sequence, and the intersequence sand in places increase greatly in thickness.

There is no evidence of any weathering interval between or within the till sequences, and thus we assume that both sequences record Wisconsinan glaciation. We believe that the lower sequence belongs to the Trafalgar Formation and the upper sequence to the Lagro Formation of Wayne (11). Wayne's plate 3 depicts a superposition of tills similar to that found in Allen County. Similar tills have also been described by Forsyth (5) near Defiance, Ohio.

The stratigraphically lowest and possibly the oldest unconsolidated materials yet examined in Allen County are exposed in the May Stone and Sand Company Ardmore Road quarry (NE¼, Sec. 29, T30N, R12E). This unit (Fig 3, Unit 1) consists of about 7 feet of medium to fine loamy sand to cobbly gravel, which, when moist, is dark gray, olive gray, and olive (5Y hue). Distinguishing characteristics of these lowest gravels are their high content of pebbles of angular chert and of igneous and metamorphic rocks, low content of shale, and lack of any carbonate except in a basal rubble layer. Clay mineral analyses show a significantly greater amount of mixed layered material than is present in samples of the area's oldest tills. The deposit rests upon Devonian limestone, possibly within a small bedrock valley, and is overlain by a generally clayey but variably textured till (correlation uncertain). It has the appearance of a weathered, reworked cherty lag deposit on an old erosion surface, but igneous and metamorphic pebbles indicate a previous or contemporaneous glaciation.

The Classic Glacial Lake Maumee Sequence

It has been supposed since 1915 that a lake level at about 800 feet was formed in the upper Maumee basin soon after the ice front retreated from the Fort Wayne Moraine (9). This lake was succeeded by

another lake at 760 feet, after which the water rose again to 780 feet (790 feet according to some sources). The Maumee stages of the pro-Erie Lobe lakes ended once lower outlets were free to discharge into the Saginaw Bay area and into the Grand River valley.

For the sake of argument we will assume that three lakes existed at roughly these elevations. However, examination of 7½-minute topographic maps not available to earlier workers shows that gross miscorrelations of beaches were made and that many of the supposed beaches are probably near-shore features other than beaches. Thus, we feel that the nature and origin of these features and their correlation and sequential order should be restudied.

Knowledge of the basic glacial stratigraphy and of this classic lake sequence now provides the basis for a discussion of the morphology, stratigraphy, and developmental history of the outlets of the Maumee glacial lakes.

Morphologic and Stratigraphic Relationships in the Wabash-Erie Channel and the Fort Wayne Outlet

Terminology

The entire valley through which glacial Lake Maumee drained, extending from just east of Fort Wayne to the Wabash Valley below Huntington, was called the Wabash-Erie Channel by Dryer (3). Leverett (8) preferred the term Fort Wayne Outlet for the same channel. Because

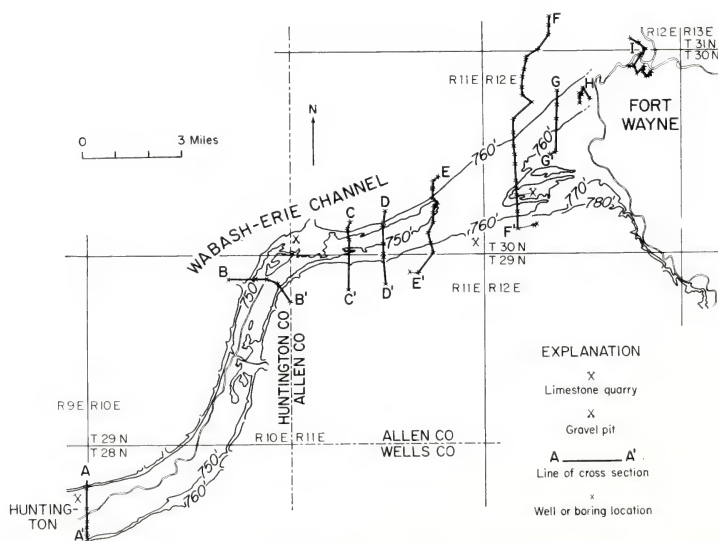


FIGURE 4. Topography of the Wabash-Erie Channel floor, showing 10-foot contours and lines of cross section referred to in text. Maximum scoured elevation of Wabash-Erie Channel in Cross Section H, 742 feet; G, 740 feet; F, 743 feet; E, 739 feet; D, 735 feet; C, 735 feet; B, 745 feet; A, 735 feet (limestone bedrock). Cross sections H, E, D, and B show 10 to 25 feet of soft clay and/or loose sand below the uppermost unit of fine-grained fill.

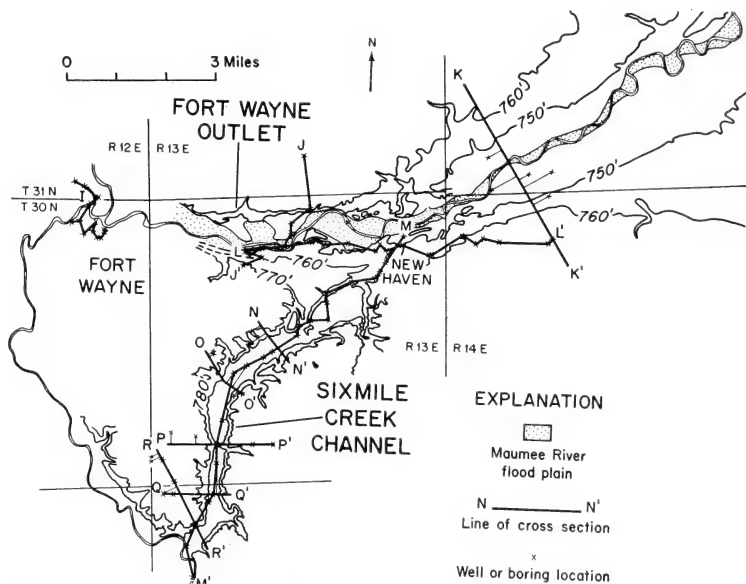


FIGURE 5. Topography of the floor of westernmost glacial Lake Maumee and the Fort Wayne and Six Mile Creek outlets, showing 10-foot contours and lines of cross section referred to in text. Maximum scoured elevations of lake floor and Fort Wayne Outlet shown in Cross Section K, 750 feet; J, 750 feet.

of their different although related origins we find it desirable to distinguish the mile-wide trough below Fort Wayne from that part of the valley that was cut through the Fort Wayne Moraine in Fort Wayne (Fig. 1). Therefore, we will use Dryer's term Wabash-Erie Channel for that single broad valley that is southwest of the city of Fort Wayne and that extends to Huntington. The head of this valley is the area of merging drainageways in the west half of T30N, R12E (Fig. 4). We will restrict the term Fort Wayne Outlet to that narrow valley extending from Sec. 4, T30N, R13E, the western point of the Maumee lake basin, westward through Fort Wayne to the point where the St. Mary's River turns east in Sec. 2, T30N, R12E to join the St. Joseph River (Fig. 5).

Morphology of the Outlet and Channel Floors

The general course of the channel between Fort Wayne and Huntington is of particular interest because of the possible indirect effect of bedrock topography upon the east-west segments (compare Valley D, Fig. 2, with Wabash-Erie Channel, Fig. 1) and because of the obvious effect of ice-controlled drainage upon the northeast-southwest segments of its course. As noted by Dryer (3), west of the outlet through the Fort Wayne Moraine "the channel turns to the southwest in a line which is a direct continuation of the valley of the St. Joseph River." Similarly, the valley also turns abruptly southwestward near the west Allen County line just west of where it bisects the Wabash Moraine and joins the continuation of Aboite Creek, which parallels the distal margin of the north

limb of the moraine. The St. Joseph and Aboite Creek drainageways, like the nearby Mississinewa, Salamonie, upper Wabash, and St. Mary's Rivers, clearly have ice-marginal or otherwise ice-controlled trends.

Dryer (3) stated that "the present level of its bottom [the Wabash-Erie Channel] rises from 737 feet at the mouth of the St. Joseph to 756 feet at the summit 4 miles west, then falls to 744 feet at the margin of the Niagara outcrop, and to 699 feet at its junction with the Wabash," although Dryer recognized that those elevations did not record the scour depth of glacial lake outflow. He recorded about 4 feet of peat overlying blue clay underlying the Wabash-Erie Channel and 40 feet of silt over limestone at the outlet summit (discussed further below). Leverett (8), however, recorded the bed of the outlet in Fort Wayne as 755 feet (misquoted as 757 feet by Hough (7)) and noted that "parts of the bed are strewn with boulders and cobblestones, . . . indicating an old scourway. The northwest part of the city . . . stands on such a stony part of the bed." Leverett apparently considered the present floor at Fort Wayne to be the original scour depth, although he did recognize that below Fort Wayne "the bed is occupied by an extensive growth of peaty material, beneath which there is fine sand" and thus "had apparently been scoured out somewhat below its present level during the most vigorous stage of the excavation and was then filled in as the strength of the flow declined." The surface elevation in the northwest part of Fort Wayne, southwest of the confluence of the St. Joseph and St. Mary's Rivers, is, indeed, between 755 and 760 feet (Figs. 1 and 4). The configuration of the contours, of course, has been greatly affected by the building of the city, and for that reason they have not been shown on parts of Figures 4 and 5.

Of special interest, however, is the form of the outlet channel east of the city, where it is cut through the moraine. The outlet valley is for the most part occupied by the Maumee River floodplain at an elevation slightly greater than 740 feet, but in Secs. 3, 4, and 5, T30N, R13E, ter-

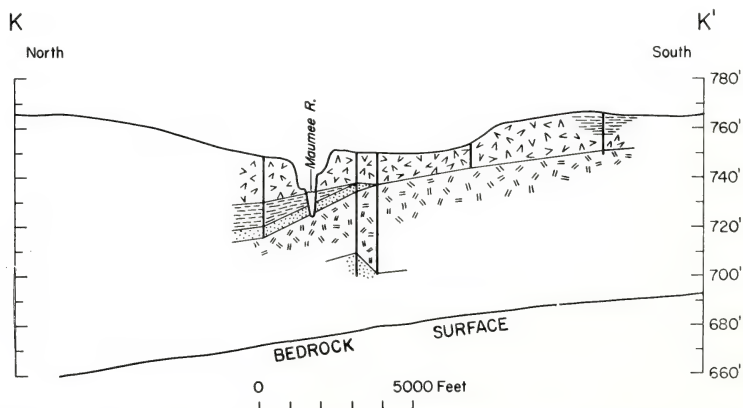


FIGURE 6. *Geologic Cross Section KK', showing geology beneath lake floor 2 miles east of New Haven. Bedrock surface interpreted from regional data. (See Fig. 5 for section location, Fig. 3 for explanation.)*

races at about 750 feet (along line of Cross Section JJ', Fig. 5) record the former scoured lake floor where it merges with the outlet.

The form of the westernmost lake bottom (along line of Cross Section KK', Figs. 5 and 6) clearly shows the constriction but also the continuation of the 750-foot contours into the outlet valley. It is clear from this geomorphic evidence that the original scoured depth in the outlet and channel downstream must have been even lower than 750 feet. In fact, this is exactly what the channel stratigraphy shows us.

Postglacial Stratigraphy within the Channel

Between 10 and 25 feet of silt, clay, and organic deposits (not yet radio-carbon dated) fill a scoured trough in the north half of the east-west part of the Wabash-Erie Channel (Fig. 7, Cross Section FF', as representative of Cross Sections CC' through H in Fig. 4). Even across the limestone sill at Huntington where Leverett (8) stated the elevation of the channel floor, and presumably the limestone surface, to be 744 feet, more than 7 feet of fine-grained fill is present over the limestone, which is at an elevation of about 735 feet in the center of the channel (along Cross Section AA', Fig. 4). Maximum elevations of the floors of the Fort Wayne Outlet and the Wabash-Erie Channel before the postlake infilling of the channel are given in the captions for Figures 4 and 5. It is clear that a significant buried trough, about the width of the valley of the Fort Wayne Outlet, occupies part of the larger Wabash-Erie Channel. The floor elevation of the buried trough, not the surface elevation, records the depth to which glacial Lake Maumee scoured. The buried channel floor appears to have broadly spaced highs and lows, whose origins are difficult to interpret. However, filling by at least the uppermost fine-grained unit must postdate the Lake Maumee discharge, and we interpret this to mean that the St. Marys and St. Joseph Rivers continued to flow sluggishly westward down the channel prior to the development of the Maumee River.

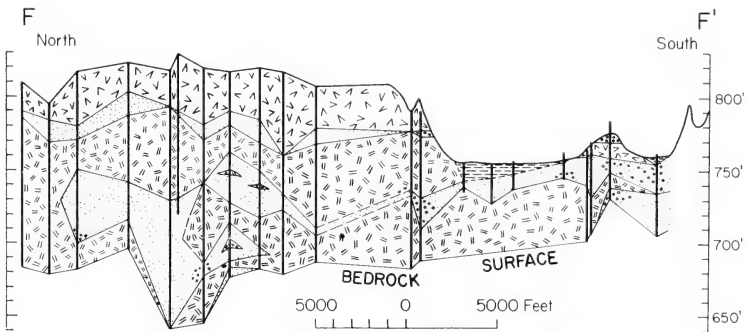


FIGURE 7. *Geologic Cross Section FF', showing representative geology of Wabash-Erie Channel and surroundings. (See Fig. 4 for section location, Fig. 3 for explanation.)*

The geology of the channel area is particularly complex. Inter-sequence sand and silt or lower sequence sand is present beneath the latest channel filling in many sections across the channel, the outlet, and the westernmost lake bottom (Fig. 7 and as along Cross Sections JJ' and

LL', Fig. 5). Because of this, it is almost impossible to assign position and age to many such deposits that occur immediately below the latest fine-grained fill. Often, only engineering data can give any clue as to whether sediments are exhumed or postglacial. Furthermore, there are several very deep holes in the channel filled with as much as 25 feet of soft (not overconsolidated) sediments to well below even the elevation of the bedrock sill at Huntington (see captions, Figs. 4 and 5).

Stratigraphic Evidence Bearing upon the Early Development of the Channel

Preliminary study of subsurface data suggests that the route of the present channel in and just west of Fort Wayne is roughly parallel to the route of drainage associated with lower sequence ice. Furthermore, two upper sequence tills, possibly representing the advance of the Erie Lobe to the Wabash Moraine and an earlier Erie Lobe advance, are separated in one major exposure on the south wall of the present channel (Midwest Aggregates quarry, Sec. 36, T30N, R11E) by a thick gravel that contains armored till balls derived from the lower till. This outwash may have been concentrated, before formation of the Wabash Moraine, along the present trend of the Wabash-Erie Channel, just as earlier meltwaters had been concentrated there.

During later development of the channel, high level outwash was deposited west of the confluence of the St. Joseph and St. Mary's valleys in continuation of the terraces in those valleys. Outwash as much as 25 feet thick is present as high as 785 feet in the west half of T30N, R12E (Figs. 1 and 4). Thus, a precursor of the present Wabash-Erie Channel was also in existence while the Fort Wayne Moraine was forming and discharging outwash through the St. Joseph and St. Marys valleys.

The lows in the channel floor that are filled with soft sediment in some way relate to the overall development of the channel. Were they cut by waters under considerable head flowing off or through ice? Do they represent plunge pools that consecutively developed as the channel lengthened? Or is there another explanation? We do not know.

But as a generalization, we can say that the Wabash-Erie Channel had a long, perhaps in part repetitive history of drainage and that most of the route as we see it today was an already established drainageway before the Fort Wayne Moraine and thus also before the Maumee glacial lakes came into existence. As noted above, the course of the main east-west segment of the channel may have been indirectly bedrock controlled, and the northeast-southwest courses were determined by ice.

Six Mile Creek Channel

Morphology

The only other incised breach in the Fort Wayne Moraine, the Trier Ditch, or the Six Mile Creek Channel as it was called by Dryer (Figs. 1 and 5) (3), connects the St. Marys River at 750 feet elevation with the Maumee River $8\frac{1}{2}$ miles away at an elevation of 740 feet (Figs. 1 and 5). In the northern one-third of its length the channel broadens considerably and the 780-foot scarp swings into the embayment. The course of this

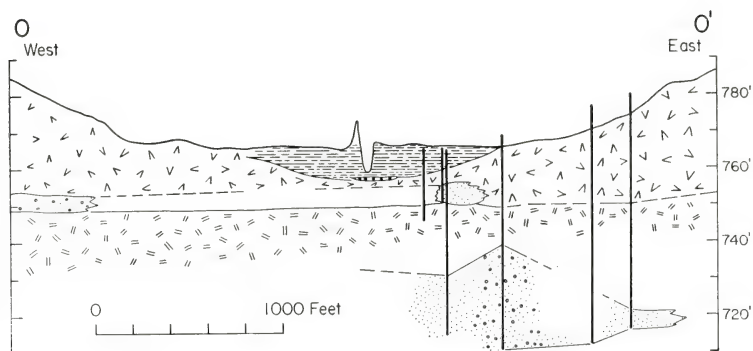


FIGURE 8. *Geologic Cross Section OO', showing representative geology across Six Mile Creek Channel. (See Fig. 5 for section location, Fig. 3 for explanation.)*

channel may have been indirectly determined by a minor bedrock valley (Fig. 2 E), which was tributary to one of the major north-flowing pre-glacial streams.

Glacial and Postglacial Stratigraphy

As elsewhere in the Fort Wayne Moraine, the stratigraphy is relatively simple and is described by the basic sequence shown in Figure 3. In the southern part of the channel a thick basal sand is overlain by hard sandy till of the lower sequence (Cross Section OO', Fig. 8, as representative of Sections NN' through RR'). This in turn is overlain by a thin dense fine sand or silt of the intersequence unit. The upper clayey till sequence lies above. All glacial stratigraphic units, as shown on Cross Section MM' (Fig. 9), drop in elevation northward as they approach the lacustrine plain and the axis of the Erie Lobe. The upper part of the lower sequence here and near the main Fort Wayne Outlet becomes much more varied where it is topographically low, containing abundant intercalated sand and silt. Lacustrine and fluvial materials associated with glacial lakes

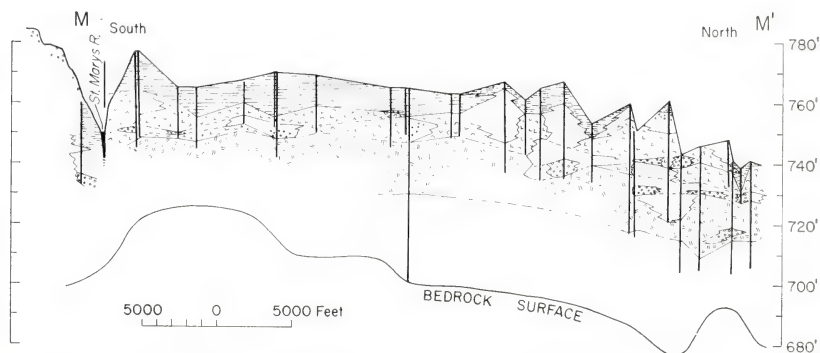


FIGURE 9. *Geologic Cross Section MM' along the Six Mile Creek Channel. Bedrock surface interpreted from regional data. (See Fig. 5 for section location, Fig. 3 for explanation.)*

and recent alluviation rest on both upper and lower sequence units along the channel, depending upon the depth of erosion.

Although there may have been a bedrock-determined initial sag, there is no indication that the Six Mile Creek Channel was active prior to deposition of the upper clayey till sequence. It appears that the contact of the upper and lower sequences may be lower there than it is a few miles to either side, but the data are insufficient to tell whether this indicates a valley or a local, closed low. The scoured floor of the present channel was cut in upper sequence clayey till at an elevation of 755 feet and, as would be expected, does seem to drop in elevation at both ends. The slope to the St. Mary's is very short and can be attributed to the more recent drainage. The slope to the north begins nearly half way along the length of the channel, is very gentle, parallels the boundary between the lower sequence till and the basal sand, and becomes difficult to trace as the stratigraphy becomes complex near the lake border. Leverett (8) pointed out that current bedding in gravels exposed southwest of New Haven shows a southward transport, which he attributed to drainage from glacial Lake Maumee. Cross beds could, of course, be related to storm deposits or longshore drift. Furthermore, it is obvious (Cross Section MM', Fig. 9) that no coarse sediment is found in the base of the channel in its southern half and that there is no appreciable buildup of such material opposite its south end. Thus, there is no clearcut reflection in morphology or general stratigraphy of the lake drainage that must have passed southward in the Six Mile Creek Channel.

As suggested by Dryer (3), the St. Mary's River used the channel as a flood stage outlet after lake levels had dropped. As recently as 1913, a flood of water 5 feet deep flowed through it. Leverett and Taylor (9) made reference to this event, and Dryer suggested that it was a frequent occurrence before the advent of modern drainage controls.

Dryer (3) defined a large sandy area in and just east of New Haven as the delta of the St. Mary's-Six Mile Creek Channel. However, the topographically prominent sand at the east end of the supposed delta appears to us to be dunal rather than deltaic, the dune sands lying upon coarse sands on the lake floor. The broadest expanse of the so-called "delta" is found in the city of New Haven where the elevation is about 760 feet, but most of the town is underlain by hard loam till.

The Six Mile Creek gap, like the Fort Wayne Outlet, was very likely an original low in the Fort Wayne Moraine. When the ice of the Erie Lobe began to recede, there were surely isolated small lakes ponded in front of it which had their own outlets. Six Mile Creek, which is now of a magnitude equal to that of the channel of the Imlay Outlet, may have been one of these. Although a large quantity of meltwater may have been spilled through Six Mile Creek Channel after integration of the ice marginal lakes into Lake Maumee, the prevailing currents and sediment transport from the lake bed were through the larger Fort Wayne Outlet. After the lake was emptied and present drainage patterns established, the Six Mile Creek Channel acted as a floodwater route for the St. Marys River.

Evidences for the Lake Sequence

Since Leverett's (8) first description in 1902 of the high stand of glacial Lake Maumee, it has been assumed that that lake drained primarily through the Fort Wayne Outlet and the Six Mile Creek Channel. Although Taylor (9, 12) had traced the uplifted continuation of the highest beach to a point 6 miles north of Imlay, Michigan, well north along the west wall of the present channel at Imlay, neither author, for some reason, concluded that the high lake drained at Imlay. It has been always recognized that the intermediate lake level drained through the Imlay Outlet, but Hough (7) pointed out that that level, too, must have drained through the Fort Wayne Outlet. Leverett (8) originally accounted for the change in level from 800 feet to 780 feet by the opening of the Imlay Outlet. If the high lake drained at Imlay, however, the opening of the Imlay Outlet alone (at least to the extent of the channel presently visible) could not explain the lowering of the lake to the 780-foot level.

The "third beach" at 760 feet was recognized in 1908 by Leverett (11) and was placed last in the sequence, although Leverett suggested the possibility that the low level might actually be intermediate in age, based upon its "rather washed-down appearance." Leverett further stated that the third Maumee beach "appears to have been barely high enough to have opened into the Imlay Outlet if its northward rise is as rapid as that of the first and second beaches." Taylor (9), however, made no mention of this observation, and assumed that the floor of the Imlay Outlet visible today, like the floor of the Fort Wayne Outlet, was too high to have drained the low-level lake.

We believe that the overriding reason for Taylor's (9) placing the 760-foot lake level between the two higher levels in the lake sequence was not the washed look of the 760-foot beaches, rather it was that the presumed lack of an outlet for the lowest lake necessitated the postulated burial of the lake's outlet by a subsequent glacial advance that also raised the level of the lake. Taylor (9) felt that a "readvance of the ice did not [completely] close the [wider and deeper 760-foot] outlet [at Imlay] but pushed it [westward] up the slope to the place where the Imlay outlet channel is now found." The head of the Imlay channel visible today was thought to have drained only the last level, the 780-foot lake.

However, we have shown that the scoured floors of the Fort Wayne Outlet, the Wabash-Erie Channel, and the Six Mile Creek Channel are deeper than previously assumed. At least 10 feet of water from a 760-foot lake could have passed through the Fort Wayne Outlet, and concurrently 5 feet of water could have passed through the Six Mile Creek Channel. Furthermore, the important limiting elevation of the limestone sill at Huntington is nearly 10 feet less than indicated by Leverett, and thus discharge gradients of all lakes were considerably more than previously assumed. Preliminary study of modern 7½' topographic maps seems to indicate that the two high beaches can indeed be traced into the Imlay channel and that taking into consideration about 50 feet of post-

glacial uplift, the 760-foot lake might also have barely discharged through the channel, just as Leverett (11) suggested.

The subdued or washed look of the 760-foot beaches has never been quantitatively documented in the literature. Many of the classic 760-foot beaches are far above that elevation, others can easily be explained as nonbeach features, and any truly subdued beach certainly can be explained in other ways than by the washing action of a higher lake. Therefore, we feel that there is no evidence for other than the simplest sequence of the classic Maumee Lake Levels, 800 feet, 780 feet, 760 feet.

Cause of Change in Level

An 800-foot lake level would have been controlled by the initial configuration of the surface of the Fort Wayne Moraine. Assuming a constant discharge rate, it is possible that the opening of a discharge route at Imlay in addition to the Fort Wayne and Six Mile Creek outlets could have balanced the lake level at a lower elevation, as Leverett (8) originally suggested. But backcutting in the Fort Wayne Outlet eventually would have cut through the moraine, perhaps in a "stoping" process of headward advance of a rapids or chute similar to that postulated for the outlet of glacial Lake Chicago by W. C. Alden (1) and modified by J. W. Goldthwait (6) in the early 1900's.

We suggest the possibility that a significant stratigraphic sill, the top of the lower hard till, could have temporarily slowed downcutting at Fort Wayne, perhaps causing a stabilization of the lake at the intermediate level. The Fort Wayne Outlet is cut into intrasequence sand and apparently into the lower hard till, which is encountered at 750 feet in borings in Fort Wayne just south of the outlet, and up to 780 feet just northwest of the city (Fig. 7). (The Six Mile Creek Channel is cut entirely in the upper clayey till.) The lowest lake level could have been initiated once erosional equilibrium was attained in the outlet and channel, or the lake could have been stabilized by a balance between glacial melting and lake discharge, perhaps due to partial opening of the drainage route that eventually lowered water to the glacial lake Arkona levels.

Acknowledgments

Compilation of these data has involved the cooperation of many individuals and organizations. Essential information has been provided by the Department of Natural Resources, Division of Water; the Indiana State Highway Department, Division of Bridges and Division of Materials and Tests; Soil Testing and Engineering, Inc., Fort Wayne; the office of the Allen County Surveyor; and the Fort Wayne Water Pollution Control Department. Stone quarry and gravel pit operators permitted repeated access to their excavations. We are grateful to all of these people and to the many Indiana Geological Survey colleagues who aided in interpretations, laboratory work, and field investigations.

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Engineering Soils Mapping in Indiana by Computer from Remote Sensing Data¹

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Abstract

Multispectral imagery of the surface terrain may be obtained from airborne scanning devices that measure reflectance in the visible through infrared portion of the electromagnetic spectrum. At the Laboratory for Applications of Remote Sensing (LARS), Purdue University, multispectral imagery is analyzed using a high-speed digital computer. The final result is a detailed map or classification of the surface material.

Pattern recognition (classification) is accomplished by separating materials according to their spectral response statistics in a known area and then applying these criteria to unknown areas. As an example of the LARS mapping technique, a detailed analysis of a soils area in north-central Indiana is presented.

In this study the correct classification of sandy floodplain soils versus till plain soils was obtained for 98+% of the ground area elements (remote sensing units) for the training fields. Similarly a 76+% correct discrimination between the two materials was made for the test fields in the flightline. This degree of accuracy indicates that the analysis of these data using the LARS techniques was successful in differentiating the materials sufficiently well to be of considerable use in reconnaissance surveys.

Introduction

Aerial photography has been used for several decades in reconnaissance mapping of soil, rock, vegetation and man-made features in a variety of scientific specialties, including geology, geography, agronomy, forestry, hydrology, civil engineering and a number of others. With the development of improved instrumentation systems for measuring electromagnetic radiation of the terrain and with the advent of high-speed computers, it is now possible to obtain more precise data which can be analyzed in great detail by digital computers.

Remote sensing involves the identification and classification of physical objects through analysis of data obtained from sensing devices which do not come in direct contact with those objects. In most cases the instruments measure radiation intensities from selected portions of the electromagnetic spectrum. For remote sensing studies that incorporate analysis by computer, the data-gathering phase is followed by some data reduction and reformatting which expedites subsequent data processing. The final phase, analysis, involves classification of the target materials through the use of pattern recognition techniques.

This paper presents a discussion of remote sensing of soil and rock materials with specific emphasis on the techniques developed at the Laboratory for Applications of Remote Sensing, Purdue University.

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Methods: LARS Automatic Classification Technique

Perhaps the first question to answer is why use an automatic classification system at all? Why not rely on the current method of mapping soil and rock for geological and other purposes. An obvious answer is that computers are faster and more accurate. Computer use does not remove the scientist from the operation; it makes him more important. As before, accurate field work is necessary; however, the same amount of field effort can now be used to map a larger area. Reliable data are needed to train the computer for proper classification but this detail is not required for the entire mapping area.

Currently, multispectral imagery of the terrain is obtained for LARS by the Institute of Science and Technology, University of Michigan, using a multispectral optical-mechanical scanner mounted on their DC-3 aircraft. As the plane proceeds along the flight path, the energy radiated by a specific ground resolution element passes through the scanner optics, is divided according to its spectral wavelength, and is directed to the appropriate detector. The output of all such detectors is simultaneously recorded by a multiband recorder. The transverse motion provided by the rotating mirror and the forward motion of the aircraft cause a continuous signal to be recorded for each spectral band of the scanner output. The information is stored in analog form by the data recording system.

The multispectral bands recorded on the aircraft storage tapes vary according to the needs of the researcher. In this study, 12 channels of data were obtained from the visible and reflective infrared portion of the spectrum, between 0.4 and 2.6 micrometers¹ (μm).

Following the multispectral remote sensing flights, the aircraft scanner analog data tapes are forwarded to LARS for conversion to digital form. Each analog scan is sampled, normally at a sampling rate which yields 220 data points for an 80° field of view across the flightline. For later reference, each digitized data point (remote sensing unit or RSU) is assigned a unique address in a two-dimensional coordinate system based on scan line numbers ("line numbers") and samples within the line ("column numbers").

Typically the first step in the analysis is to obtain gray scale printouts of the data for several channels. Gray scale printouts are digital displays of the spectral response of the terrain but limited to one band or channel of the scanner data per display. They resemble low-resolution photographs. Alpha-numeric symbols (letters of the alphabet, typing symbols and single digit numbers) are assigned to the radiance levels so that high-intensity or light areas receive symbols which cover a low percentage of the paper (Example ---), and dark areas receive symbols with a high percentage of cover (Example MMM). Because the data are not equally distributed between the highest and lowest radiance values, the symbols are not simply assigned so that each represents an equal range of radiance. Instead, to achieve a maximum contrast display, the data

¹One micrometer (μm) = 10^{-6} meters.

are first histogrammed and the symbols are assigned so that each will occur with approximately the same frequency.

Areas of known materials are located on the gray scale printouts by the researcher and their addresses are recorded. This information, referred to as "ground truth," is used to train the computer to recognize similar kinds of material. In agricultural studies, this may consist of fields containing corn, wheat, oats, soybeans, etc.; in forestry studies—conifers and deciduous trees or individual tree species; in geology and highway engineering—specific bedrock and soil types. While some of the ground truth areas are used to train the computer to recognize the classes of interest, the remaining areas are reserved for testing the accuracy of the computer classification after it is completed. Addresses of training areas are provided to the computer on punched cards; these addresses are boundary corners indicated by column and line number, and the fields are limited to rectangular shapes (commonly referred to as "fields").

After January 1, 1971, an alternative to using gray scale printouts for field selection became available at LARS. This tool, known as the digital display unit, provides a television-like image of the scanner data with each digital value represented by a different brightness level. This yields an image on the television screen having greater detail than is possible with gray scale symbols on computer paper. Images for each channel can be displayed, and fields of interest can be outlined on the screen using a light pen with their addresses automatically punched on cards.

Another method for obtaining training field sites is sometimes used in conjunction with the above procedure of manually selecting these sites. Accomplished by using the LARS non-specified classifier routine (NSCLAS), this relatively new technique divides the scanner data into groups or clusters based on similarity of spectral response within clusters. Typically 4 to 6 channels of imagery are analyzed simultaneously and the program is requested to obtain 10 clusters. A map of the results is printed for each area analyzed in this way and the researcher can observe the patterns of spectrally differentiable material occurring in the data. The training fields may then be selected from these maps. The resulting clusters may not in fact be spectrally distinct, but the researcher must decide this to his own satisfaction based on separability information for the clusters which is printed by the program. He then has the option of repeating the analysis using a different number of clusters to increase their separability. An important point is that this method of selecting training fields takes into detailed account the multispectral response data as well as the ground truth information.

In the clustering approach, the actual differences in spectral response are displayed, but a major problem exists in determining what each of the clusters represents. Some may be vegetation types, some water bodies, man-made features or tonal aspects of bare soil and rock. Aerial photography is helpful in affixing names to the patterns observed. Despite this difficulty, clustering is a powerful tool in obtaining workable training fields.

The next step in the analysis is to obtain histograms of each class of material identified in the training fields. An example of classes for a geologic study area might be alluvium, limestone soil, shale soil, trees, mixed crops, and water. The histograms for each class show the distribution of reflectance intensity for each spectral channel.

Unimodal or single-peaked distributions in the histograms for a class suggest that the proposed class is spectrally an individual group. Bimodal or trimodal distributions must be subdivided manually into unimodal classes by the researcher; histograms for individual fields can be obtained to help in locating the multimodal contribution within a class.

The next operation involves the application of a divergence (statistical separability) analysis (known as \$DIVERG) to determine the best channels to use for classification. Only the best four to six channels are used for classification to save computer time; in general, the accuracy is not meaningfully increased when more channels are added. In addition to indicating the preferred channels, the divergence analysis indicates the separability of the designated classes. If separability between significant materials is poor, some of the preceding steps are repeated in an attempt to improve this separability and hence classification accuracy.

Next, training field statistics are used to classify the designated portion of the flightline. The computer classifies each data point (RSU) based on the maximum likelihood criterion. On request, the computer calculates how accurately it classified the areas used for training by comparing the classification of each point in the training fields with the initial ground truth designation. A high level of agreement means there is minimal confusion within the training field statistics for the various materials and that the classes are being separated properly.

The final steps are to print out a computer classification for the whole area and to determine how well the test fields were classified. The test fields are those areas of known material from ground truth studies that were not previously used for training purposes. If test fields and training fields show a high degree of accuracy, and the test fields are representative of the entire area, the classification is a good one. If the accuracy is low, some reworking of the classes should be done. The researcher may also have to conclude that the classes involved are not spectrally separable.

Results: Tippecanoe County, Indiana, Study Area

To illustrate the automatic classification technique for engineering soils mapping, a flightline in Tippecanoe County, Indiana, was selected for study. The test site known as Tippecanoe County Flightline 23 is a north-south line running approximately through the center of the county, including a central strip through the Lafayette-West Lafayette metropolitan area along the Wabash River (Fig. 1).

Scanner data were obtained for Flightline 23 during various seasons of the year in 1969 and 1970 for the on-going LARS program of study of the midwest corn belt area. Seasonal changes of soil patterns and crop

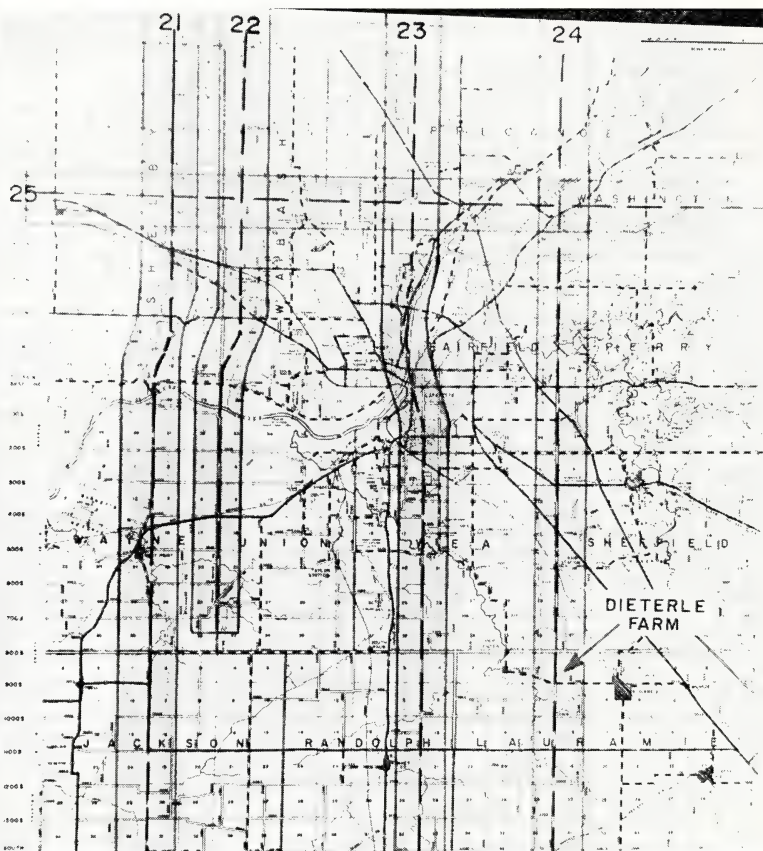


FIGURE 1. *Tippecanoe County, Indiana, flightlines.*

cover were observed by these periodic flights over the established flightline. In the current study, the scanner flight dated May 6, 1970, was selected for analysis. In general, spring flights are best for soil classification studies as the maximum percentage of bare soil is exposed at this time of year.

Analysis was limited to the northern third of the flightline which contains examples of the soil materials of greatest interest in the study. The northern 5 miles of the flightline cover a portion of the Tipton Till Plain. South of this segment is a 2-mile section which runs across the Wabash River floodplain. These two sections comprise contrasting parent materials and form the basis for the soils study of the 7-mile area. Flown at an elevation of 3,000 feet, the flightline has a width of about 5,000 feet.

The initial step in the analysis technique was to compare a gray scale printout of the flightline to black and white photography taken at the time of flight. The Wabash River was outlined, as were the floodplain

and till plain areas and several significant man-made features; the By-Pass 52 bridge and Interstate 65, which was under construction at the time of flight.

To determine the material types present in the study area, 13 east-west strips of data were taken at various points of interest along the flightline. An NSCLAS was run on these combined transverse strips which collectively comprised nearly the maximum number of data points that the routine can handle.

In the 13 clustered strips, vegetation, water, man-made features and bare soil were identified with the aid of the aerial photography. Rectangular fields were selected in the bare soil areas and a second NSCLAS was run on these combined areas using ten clusters for the analysis. This yielded a numerous collection of rectangular fields with soil patterns designated in each.

By selecting those fields (or portions of fields) which had predominantly one pattern, a collection of bare soil fields, each of essentially one soil type, was assembled. Two distinct groups were maintained, however: those fields from the floodplain area and those from the till plain.

Next, statistics were obtained for each field and the histograms examined to insure that each was unimodal. Three divergence analyses (\$DIVERG) were made on the fields to determine how to combine them into separable classes. Two runs were required initially because the programs accept a maximum of 30 fields in the divergence analysis and the 50 fields were divided into two groups to accomplish this. Fields deemed similar by the divergence analysis were combined, whereas, others were left by themselves and a few confusing fields discarded. After this was achieved for the two divergence runs, the remaining groups (numbering less than 30) were collected in the final divergence analysis and the combining technique repeated. The net effect is that 18 classes remained after this compilation, 8 from the floodplain area and 10 from the till plain.

Several classifications were made of the 7-mile segment using the training statistics from these classes. Adjustments were made in successive classifications to improve the training field performance. In the final classification this performance was sandy floodplain 99.1%, and till plain 98.5%. These values are somewhat misleading as a considerable number of floodplain symbols were scattered within the till area although the till symbols are rather minimal in the floodplain area.

To obtain a more meaningful indication of the classification's accuracy, a large number of bare soil fields were selected as test fields. In the sandy floodplain, 28 test fields containing a total of approximately 1,900 RSU's and 59 test fields in the till plain (approximately 8,700 RSU's) were included. Test results showed 92.7% accuracy for the sandy floodplain and 76.8% accuracy for the till plain with an overall accuracy of 76.8%.

Because of limitations on the size of figures and their number in this paper, the computer printout, which is quite large and loses much detail in photo reduction, is not included here.

An intriguing situation was noted during the classification revisions. The till plain areas most often confused with floodplain soils were in recent road cuts where reflectance of the till was the greatest. This was observed near the By-Pass 52 bridge cut on the west side of the Wabash River and for borrow and fill areas along Interstate 65.

An interesting substudy was made on the flightline as a final effort. Interstate 65 runs diagonally NW-SE across the flightline. Experience at LARS has indicated that straight line, man-made features not oriented essentially parallel or perpendicular to the flightline are difficult to map in detail. Diagonal features of this sort run both away from the center of the flightline yielding changes in viewing angle from the aircraft, and up or down the flightline simultaneously. Also, as remote sensing units measured on the ground are square, the units along the pavement boundary have an averaged value of reflectance for both pavement and soil. If training samples are taken from this boundary portion, erroneous pavement symbols will appear in soil areas in other locations of the classification.

To master this problem, we clustered (NSCLAS) rectangular areas across the highway from the southeastern edge to the northwestern edge of the flightline. Ten cluster groups were designated. The pavement, median strip and shoulders were outlined in the clustered map results. As might be expected, the same cluster group was not associated with the pavement in the center as at the edges of the flightline, but the contrast of pavement and soil was apparent at each location. To insure proper classification, pavement classes from the middle, quarter point and edge of the flightline would be included for accurate designation of the highway in an overall classification.

Status of Inter-agency Erosion and Sediment Studies in Indiana

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Abstract

Indiana streams erode, transport, and deposit a variety of sediments. About 10 years ago an inter-agency committee was formed to accelerate investigations of these sediments. This committee consists of representatives from federal and state agencies. Recent fluvial sediment studies on selected streams indicate a range in sediment yield of from 30 to 880 tons annually per square mile of drainage area. Studies of sediment deposited in selected reservoirs indicate that the area draining into these impoundments is eroded at rates of from 3 to 22 acres annually. The reservoirs studied are losing storage capacities at the rate of from 0.1 to 2.9 percent annually.

Water-transported sediments have been the subject of study in Indiana for many years. Observations of fluvial sediments—those in transit in flowing streams—date back at least to 1906. Early studies consisted of observations only and included no precise measurements, however (3).

Impounded sediments—those relatively stationary in natural lakes and reservoirs—have been measured since about 1930. The first such recorded measurements were made on Winona Lake and Tippecanoe Lake, both natural lakes formed during the Pleistocene Epoch or ice age (3).

Winona Lake is estimated to have decreased in depth from 127 feet to 79 feet from the time it was left by the glacier to 1930. In this period of time the surface area and volume of Winona Lake were reduced 37.4 and 43.6%, respectively. During this period the maximum depth of Tippecanoe Lake decreased from 164 feet to 123 feet, and the original surface area and volume were reduced 20.8 and 31.9%, respectively.

Recent fluvial sediment studies have been carried out cooperatively by the Geological Survey of the United States Department of the Interior (USGS) and the Indiana Department of Natural Resources (IDNR). The first of these studies began in 1953 on the St. Mary's River near Ft. Wayne (1). The location of the suspended sediment sampling stations are shown in Figure 1, and Table 1 lists available data obtained from these studies.

The fluvial sediment studies show that each square mile contributes from 30 to 880 tons of sediment annually. The size of drainage area that contributes to the measurement sites varies greatly. The larger the drainage area the smaller the proportion of transported sediment that reaches a measuring point. The fluvial sediment studies are of insufficient duration, however, to provide more than a general estimate of the average annual sediment discharge for the streams.

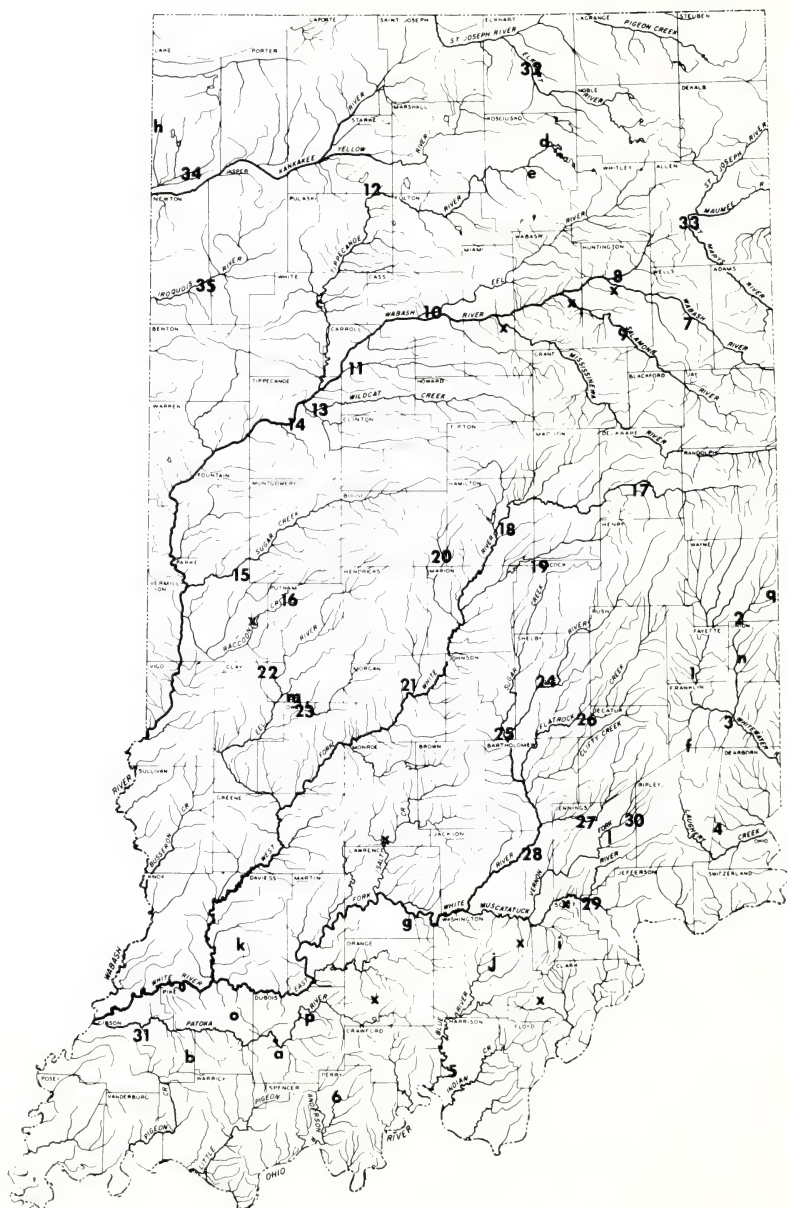


FIGURE 1. *Indiana sediment survey stations.*

About 10 years ago several interested agencies and organizations banded together to form a rather loosely organized inter-agency committee to study erosion and sedimentation in Indiana. This committee proposed two distinct types of studies, comprehensive studies and limited studies.

TABLE 1. *Average annual suspended sediment discharge from some Indiana streams.¹*

Map No.	Station Name	Type of Record	Period of Record	Drainage Area (Sq. Mi.)	Sediment Tons/yr./mile ²	Bed ² Load
1.	Whitewater R. near Alpine	Periodic	7-68 to date	529	242	S
2.	E. Fk. Whitewater R. at Abington	"	4-67 "	198	172	"
3.	E. Fk. Whitewater R. at Brookville	"	10-63 to 6-68	380	250	—
4.	S. Hogan Cr. near Dillsboro	"	7-69 to date	38.2	—	M
5.	Blue R. near White Cloud	"	7-68 "	461	49.2	"
6.	M. Fk. Anderson R. at Bristow	"	3-64 "	41.9	122	"
7.	Wabash R. at Bluffton	"	7-68 to 10-71	532	209	—
8.	Little R. near Huntington	"	7-69 "	263	357	—
9.	Salamonie R. near Warren	"	10-63 "	425	184	M
10.	Eel R. near Logansport	"	7-68 " 8-69	789	92	"
11.	" " "	Daily	8-69 " date	—	—	—
12.	Deer Creek near Delphi	Periodic	7-69 "	278	308	S
13.	Tippecanoe R. near Ora	"	7-68 "	839	53	"
14.	Wildcat Creek near Lafayette	"	7-68 "	791	161	"
15.	Wabash R. at Lafayette	"	7-63 " 7-78	7,247	286	—
16.	Sugar Creek near Byron	"	7-68 " date	668	208	M
17.	Big Raccoon Cr. near Fincastle	"	8-59 "	132	877	S
18.	White R. at Muncie	Daily	1-68 " 2-69	241	82	—
19.	White R. near Noblesville	Periodic	7-68 "	828	—	S
20.	Fall Creek near Fortville	"	7-63 " 7-68	169	73.4	—
21.	Eagle Creek at Zionsville	"	7-69 " date	103	316	M
22.	White River near Centerton	"	7-63 " 7-68	2,444	134	—
23.	Big Walnut near Reelsville	"	7-69 " date	326	235	S
24.	Mill Creek near Catawact	"	7-69 " date	245	395	"
25.	Big Blue R. at Shelbyville	"	7-68 " date	421	225	M
26.	Sugar Creek near Edinburg	"	7-68 "	474	124	"
27.	Flatrock R. at St. Paul	"	7-69 "	303	229	M
28.	Sand Creek near Brewersville	"	7-69 "	155	428	"
29.	East Fk. White R. at Seymour	"	7-56 "	305	200	"
30.	Muscatatuck R. near Deputy	"	7-68 "	2,341	147	S
31.	Brush Creek near Nebraska	Periodic	7-63 " 7-68	293	608	M
32.	Patoka River near Princeton	"	7-63 " 7-68	11.4	103	—
33.	Elkhart River near Goshen	Periodic	7-63 " 7-68	815	29.6	—
34.	St. Marys River near Ft. Wayne	Daily	5-53 " 11-67	762	216	—
35.	Kankakee River at Shelby	Periodic	7-63 " 7-68	1,753	75	—
	Iroquois River near Foresman	"	7-68 "	452	97	S

¹Data supplied by the U.S. Geol. Surv. and Indiana Dept. of Natural Resources.

Data from this table should be used in conjunction with the report by L. E. Johnson, 1970.

²M—minimal; S—possibly significant.

The comprehensive studies were to interrelate many environmental factors related to erosion and sedimentation. Such disciplines as biology, geology, meteorology, soil conservation, hydrology and sedimentology were to be involved in each watershed and lake study. The limited study was planned to include the measurement of sediment in a lake and a study of the erosion taking place in the area draining to the lake.

Unfortunately, no comprehensive studies have been made because of lack of funds and personnel, but numerous limited studies have been undertaken.

The inter-agency committee includes representatives of the U.S. Geological Survey, Indiana Department of Natural Resources, U. S. Army Corps of Engineers, the U. S. Department of Agriculture's Agricultural Research Service (ARS) and Soil Conservation Service (SCS), the Water Resource Research Centers at Indiana University and Purdue University and the Indiana Department of Natural Resources' Geological Survey (IGS).

The major effort of the committee has been in arranging for and conducting reservoir sediment studies. To date studies have been made at 15 reservoirs in the state. Locations of these reservoirs are shown in Figure 1, and pertinent data obtained from these studies are given in Table 2. The procedures employed in these studies have been previously reported (2).

The results of the reservoir surveys indicate soil losses ranging from 3 to 22 tons annually, and losses of original storage capacity of from less than 0.1 to 2.9% per year. As in the case of the suspended-sediment studies, data are insufficient to allow complete evaluation.

Additional reservoir studies are being planned, and resurveys are anticipated. It is normal procedure to allow a 10-year period after construction of a reservoir before the first sediment survey. The Corps of Engineers has installed, or is in the process of installing sediment ranges on Lakes Huntington, Salamonie, and Mississinewa. Continuing studies are being made on Cataract, Mansfield and Monroe Lakes by the Corps. The IDNR has installed sediment ranges on Deems Lake in Clark County and on the Quick Creek Reservoir in Scott County. The SCS has recently completed a sediment survey on Lake Salinda, a water-supply reservoir for Salem, Indiana (Table 2). Sediment ranges have been installed by SCS on the Elk Creek fish and game reservoir in Washington County and on the French Lick Creek fish and game reservoir in Orange County.

At the time of this writing, two Purdue University students, L. J. Lund and Manuel Poulet, are preparing a paper on some pedologic features of reservoir sedimentation. This study involves 14 Indiana and Illinois reservoirs and their drainage areas.

The long-range plan of the committee calls for resurvey of most of the reservoirs after ten years of additional sedimentation, or more frequently if conditions warrant. Ideally, a survey should be made immediately before and after a large sediment producing storm. Such storms usually occur at ten-year intervals.

TABLE 2. Summary of reservoir survey data.

Reservoir ¹	Nearest Town	Year Built ²	Surface Area in Acres	Drainage area in Sq. Mi.	Years Between Surveys	Original Storage Capacity Acre-feet	Annual Yield Tons/Sq. Mi.	Capacity Loss %/Year	Agency
Huntingburg (a)	Huntingburg	1894	24.5	0.67	46.3	137	537	0.28	S.C.S.
Oakland City (b)	Oakland City	1921	80.0	0.52	43.9	851	3897	0.11	S.C.S.
Lake Shafer (c)	Monticello	1923	1291.0	1700.00	28.0	15486	38	0.16	S.C.S.
Tippecanoe Lake (d)	Oswego	1930	1133.0	118.00	5.0	39197	No Data	No Data	I.U.
Winona Lake (e)	Warsaw	1930	562.0	32.10	5.0	27492	No Data	No Data	I.U.
Mollenkramer (f)	Batesville	1930	40.0	6.53	20.2	208	870	2.00	S.C.S.
Spring Mills (g)	Mitchell	1938	28.0	15.03	9.9	178	1420	2.90	S.C.S.
Bodeman Res. (h)	Brunswick	1947	10.7	2.73	13.0	52	284	0.01	S.C.S.
Scottsburg Lake (i)	Scottsburg	1949	92.0	3.13	12.0	749	1357	0.40	S.C.S.
Lake Salinda (j)	Salem	1949	112.0	5.70	21.0	891	1310	0.59	S.C.S.
Graham Lake (k)	Washington	1950	20.0	0.34	6.0	105	105	0.07	S.C.S.
Brush Creek (l)	Butlerville	1953	186.0	14.30	9.6	2067	1643	0.97	U.S.G.S.
Catawact Lake (m)	Cloverdale	1953	4840.0	295.00	9.2	788	742	0.01	Army Engr.
Whitewater Lake (n)	Liberty	1954	180.0	19.29	8.8	3590	1666	0.55	S.C.S.
Jones Pond (o)	Otiwell	1954	1.9	0.03	1.5	11	2972	0.67	S.C.S.
Beaver Lake (p)	Dubois	1955	200.0	3.95	9.0	2486	3023	0.34	S.C.S.
Richmond Lake ⁴ (q)	Richmond	1961	175.0	48.14	6.5	3250	725	0.99	S.C.S.

¹Letters refer to site locations on Figure 1.²Time of first study if a natural lake.³Estimated that 67% of drainage area indirect—through sink holes.⁴Richmond water supply reservoir, Richmond, Indiana.

The Indiana Inter-agency Committee on Erosion and Sedimentation has provided much useful data, but its work is just beginning. Any and all interested groups or agencies, not already involved, are encouraged to join the committee's activities.

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Precambrian Geophysical Provinces in Indiana

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Abstract

Regional statewide gravity and magnetic surveys in Indiana reveal large areas of high and low intensities. The source of these variations are lithologic changes within the Precambrian basement. A generalized map of basement provinces, constructed from both gravity and magnetic data, identifies regions of basement lithologies as either dense or magnetic-rich rocks or combinations thereof.

Analyses of intense, magnetic anomalies reveal the basement surface to vary from 2,000 to 11,000 feet below sea level. Many of the anomalies appear to be concentrated along the Cincinnati Arch. Detailed studies of two regions suggest that the sources may be basaltic pipe-like intrusions into a granitic country rock. Lava flow structures may be associated with the individual pipes.

Introduction

Geophysical surveys today constitute an important and integral part of almost every modern effort at geologic exploration. For many years the petroleum industry has routinely utilized seismology, gravity and magnetic methods in the search for oil beneath both the land and sea. However, the cost of geophysical surveys in the past have limited their use to major companies. With advances in technology, the cost of such surveys is not necessarily prohibitive. This paper summarizes data that are applicable to the study of Precambrian basement in Indiana and emphasizes regional interpretation based on the most recent gravity and magnetic studies.

Gravity Data

The first extensive gravity survey in Indiana (Fig. 1A) was conducted by the Indiana Geological Survey during 1951 and 1952 (3). Control was based upon a grid density of approximately one station for each congressional township. Elevation and locations were determined from several sources including U. S. Coast and Geodetic and U. S. Geological Survey bench marks, U. S. Geological Survey topographic maps, U. S. Engineering maps and Indiana State Highway Commission maps.

Contours show equal values of Bouguer anomaly relative to an arbitrary datum with an interval of 5 milligals. Bouguer corrections were based on an average density of 2.6 gm/cm³ for the sedimentary rocks above sea level. In addition to the statewide gravity survey, the Indiana Geological Survey and the Department of Geology at Indiana University have conducted detailed surveys over selected anomalous regions in Hamilton, Pulaski, Newton, Randolph, Wayne and Fayette Counties. These data are available on file with the Department and the Survey.

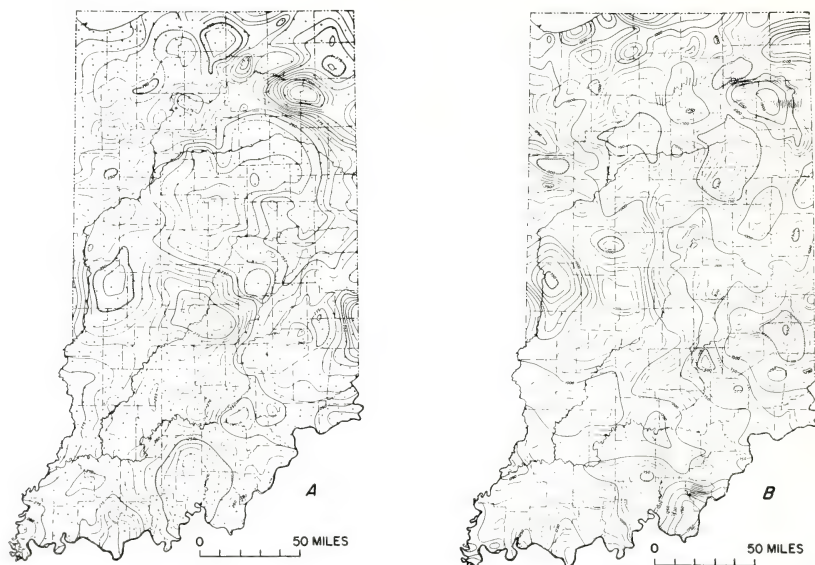


FIGURE 1. A) Map of Indiana showing gravitational intensity (3). Contour interval 5 milligals relative to an arbitrary datum. B) Map of Indiana showing average aeromagnetic intensity (4). Contour interval 50 gammas relative to an arbitrary datum.

Magnetic Data

From 1949 to 1952, the United States Geological Survey, in cooperation with the Indiana Geological Survey, conducted a statewide aeromagnetic survey of Indiana. Ninety-two county maps were published on a scale of $1''=1$ mile (1:63,360) with a contour interval of 10 gammas (0.0001 oersteds). In 1958, Henderson and Zietz compiled the county maps into a statewide map on a scale of $1''=8$ miles (1:500,000) (Fig. 2). Contours represent equal values of the total magnetic intensity at an elevation of approximately 1,000 feet above the surface. Contour interval is 50 gammas relative to an arbitrary datum.

Comparison of the aeromagnetic and gravity maps is difficult because of the discrepancy in control between the two maps. The gravity map, based on few control points, emphasizes broad scale regional features; the aeromagnetic map with continuous north-south control at 1-mile east-west flight line intervals shows great detail and thereby obscures broad scale features.

In 1953, an average magnetic intensity map of the state was compiled from the aeromagnetic data (4) (Fig. 1B). Control was based on average values obtained for the mid-point of each township by computing the arithmetic average of the magnetic intensity at the corners, the mid-point of each side, and the center of each township. Average values were further reduced by removing a south to north earth dipole gradient of 6.5 gammas per mile. Contour interval is 50 gammas relative to an arbitrary datum. Station density and contour intervals for the average mag-

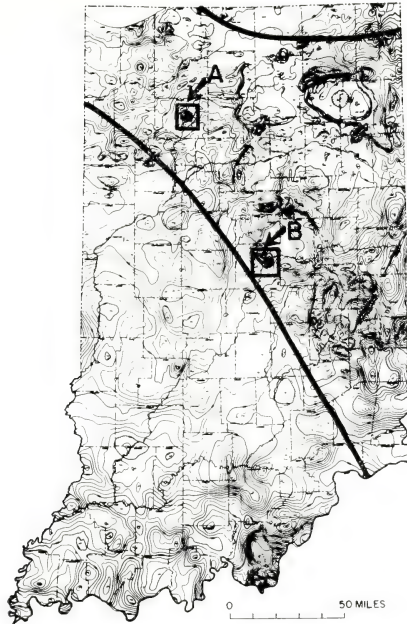


FIGURE 2. Map of Indiana showing total aeromagnetic intensity (2, Plate 4). Solid heavy lines show the approximate position of the Cincinnati Arch. Area A outlines the Pulaski County anomaly. Area B outlines the Hamilton County anomaly.

netic intensity map are comparable to the gravity map and allow comparative interpretations to be made.

In recent years, the Indiana Geological Survey and Department of Geology at Indiana have conducted detailed magnetic surveys in Hamilton, Pulaski, Newton, Wayne and Fayette Counties.

Analysis of Gravity and Magnetic Data

Lithology

Regional studies of the statewide gravity map (Fig. 1A) and average aeromagnetic map (Fig. 1B) begin with two simple assumptions:

- 1) The source of the anomalous gradients on both maps arise from lithologic changes within the basement complex (the top of the basement is 2,000 to 11,000 feet below sea level). The anomalies are not an expression of topographic relief on the basement surface. Lateral changes in density and magnetic susceptibility in the overlying sedimentary section are also not sufficient to create the anomalous contour patterns displayed on these maps.

- 2) Areas of high gravity values are underlain by dense rocks within the basement. Areas of high magnetic values are underlain by rocks with a relatively high content of magnetite (an iron oxide mineral common to certain igneous and metamorphic rocks).

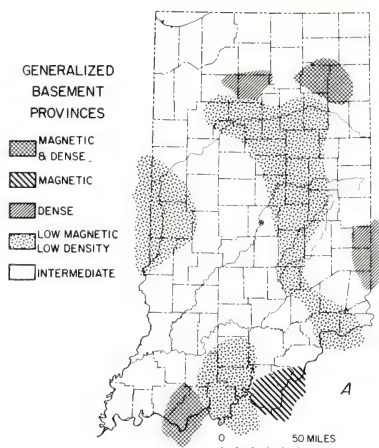


FIGURE 3. Map of Indiana showing Precambrian provinces derived from gravity and aeromagnetic data.

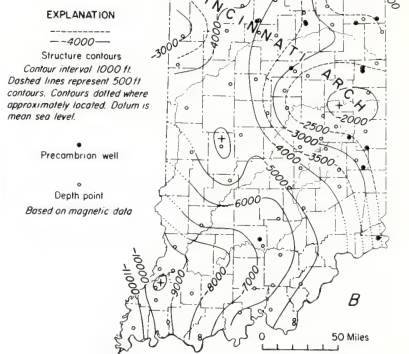


FIGURE 4. Map of Indiana showing structure on top of the Precambrian basement. (modified from (2), Fig. 11).

With the above assumptions, we have studied the statewide maps (Fig. 1) and generalized areas of extreme high and low values. Figure 3 identifies areas of basement lithologies associated with either dense or magnetic rocks or combinations thereof. For example, the stippled pattern throughout the central part of Indiana (and isolated areas on the western and southern edges of the state) delineate areas of low density-low magnetic rocks. This combination could represent metamorphosed sediments, or, perhaps a weathered granitic terrain. Magnetite-rich dense basalts (the cross-hatched pattern) could underlie the magnetic and dense areas to the northwest and southwest. A magnetite-rich zone of granitic rocks would explain the strong magnetic region in southwest Indiana (heavy diagonal pattern) and a very basic, but magnetite-poor lithology might produce the isolated region in north central Indiana (light diagonal pattern).

Studies of lithologies in the midwest (7) indicate that the average Precambrian terrain may be granitic in nature and we suggest that the areas labeled as "intermediate" basement rock in Indiana is a granitic country rock.

Depth

Studies of individual gravity and magnetic anomalies can yield estimates of a minimum depth to the top of the lithologic source of the anomaly. Henderson and Zietz (2) used 79 isolated well defined magnetic anomalies for depth calculations. Combining their results with seven deep wells, they constructed a generalized contour map of the basement surface (Fig. 4). Since the original work by Henderson and Zietz, there have been 12 additional basement wells drilled and extensive seismic reflection

coverage (5, 6). None of these data have been incorporated into Figure 4.

There are several recent publications which represent the Indiana basement surface to be a smooth topographic surface (*e.g.*, 1); it is our belief that the differences between the Henderson and Zietz interpretation and more recent versions are primarily due to lack of control. Recent maps use only deep test wells and these are poorly distributed, generally confined to the central part of the state along the Cincinnati Arch. The basement map based on magnetic analysis depends on numerous, widely distributed control.

Geometry of Basement Intrusives

We have earlier suggested that the source of many of the gravity and magnetic anomalies be within the Precambrian basement. Various rock types intruded into the granitic country create a lithologic contrast responsible for the observed anomalies. It is a curious observation that many of these anomalies are concentrated along the Cincinnati Arch (outlined by the heavy lines in Figure 1). Detailed studies of two of these anomalies have been moderately successful in defining the geometric and lithologic sources.

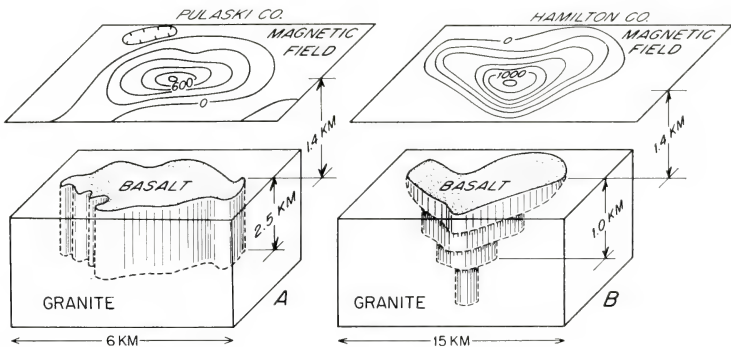


FIGURE 5. Schematic representation of Precambrian bodies interpreted as sources for the gravity and magnetic anomalies observed in A) Pulaski and B) Hamilton Counties.

Area A in Pulaski County (Fig. 1) is an isolated, well-defined anomaly approximately circular in form (Fig. 5A). Analysis of seismic, gravity and magnetic data (8) suggest that the source is a vertical, prismatic body of basaltic material intruded into a country rock of granite. Area B in Hamilton County (Fig. 1) is also a well-defined anomaly, but more complex in its form (Fig. 5B). In a recent study we suggested that the source of this anomaly is a vertical pipe, with a series of overlying flow-like sheets of basalt (9).

Viewing the interpretation of these two anomalies as part of a larger geologic picture, the Cincinnati Arch appears to be the site of numerous intrusive pipes, now represented by a concentration of magnetic anomalies. These intrusions were sources for flow-like basalts that covered the

Precambrian surface in isolated patches. We encountered flow-like basaltic rocks in several of our basement tests and this interpretation thus seems geologically reasonable. The age of the intrusions and flows is questionable, but it is suggested that they may be Keweenawan related.

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History of Brick Manufacture in Indiana¹

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Abstract

In Indiana bricks were commonly burned at the construction site in the first decades of the 19th century, but by 1840-1860 centralized brickyards were operating in or near most communities. Pennsylvanian shales and underclays, Mississippian and Devonian shales, leached glacial drift and alluvium, and residual soils have supplied the raw materials for common brick, face brick, paving brick, and firebrick manufactured during the 19th and 20th centuries.

The brick industry has progressively centralized to fewer but larger installations concentrated near particularly favorable shale and clay supplies. By 1971 less than 200 million bricks were manufactured in only 12 plants in Indiana, but the annual value of the product had grown to nearly \$8 million. This stable industry will continue indefinitely to be a significant factor in Indiana's economy.

Introduction

Indiana has long had a flourishing ceramics industry that has contributed substantially to the economic growth of the State. Plants producing building bricks have added aesthetic quality to Indiana towns and landscapes (Fig. 1).



FIGURE 1. *Andrew Wylie house, built in Bloomington in 1835 of brick fired from local clay.*

¹Publication authorized by the State Geologist, Department of Natural Resources, Geological Survey.

Little was recorded concerning the early history of the brick industry in Indiana. In 1833, the second edition of Scott's *The Indiana Gazetteer* (18) had only one description of brick clay, although brick buildings were recorded in nearly every community. Much of the brick for major structures, small groups of buildings, and isolated localities was burned at the site by itinerant craftsmen who specialized in this skill. The magnitude of brick manufacture may be judged from Scott's description (18) of Charlestown, in Clark County:

"The public buildings are a court house, a jail, an office for the clerk and recorder, and a market house, all of brick; in addition to which the Episcopal Methodists, the Reformed Methodists, the Baptists, and the Presbyterians, have meeting houses, all of brick, and an extensive brick building has lately been erected for the purpose of a county seminary . . . There are in Charlestown, about sixty-five brick dwelling-houses, and about a hundred of wood."

It is known that by the 1840's and 1850's almost every community in the state had a brick yard that manufactured common brick (11). Some clay deposits and ceramic plants were briefly identified by early authors ranging from David Dale Owen in 1839 (17), through Cox (9, 10), and Collett (7, 8), to Maurice Thompson in 1886 (19), and W. H. Thompson in 1889 (20), but these reports were short and offered little basic information on the extent and geology of the deposits and the facilities at the factories.

In 1895 the newly appointed State Geologist, W. S. Blatchley, began to gather information concerning the clay and shale resources of Indiana.

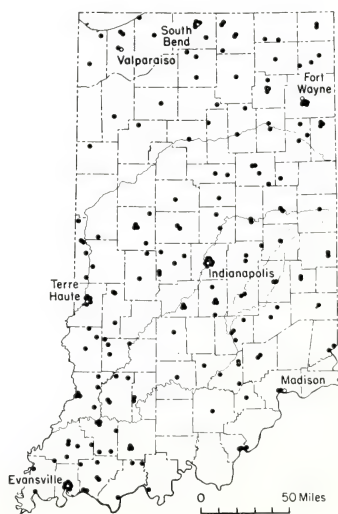
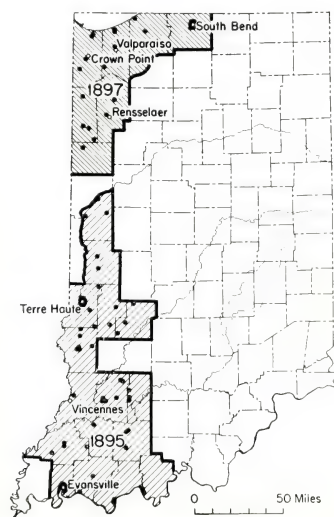


FIGURE 2. Brick plants of southeastern Indiana in 1895 (2) and of northeastern Indiana in 1897 (3).

FIGURE 3. Brick plants of Indiana in 1904 (4).

This inquiry led to the 1896 publication of a report (2 and Fig. 2) on the clay and related industry of some counties in southwestern Indiana. Two years later a report on the clays and the clay industry of northwestern Indiana followed (3 and Fig. 2). Blatchley's work on clays culminated in the publication, in 1906, of a very extensive report on the clays of the entire state (4 and Fig. 3).

Logan (15) presented some information about the clay industry in the *Handbook of Indiana Geology*, published in 1922, and in 1933 Whitlatch (21 and Fig. 4) published a comprehensive study on the clay resources of the state. Whitlatch's report, which was his Ph. D. dissertation, updated Blatchley's information on the clay industry and also emphasized the regional geology of the clay deposits.

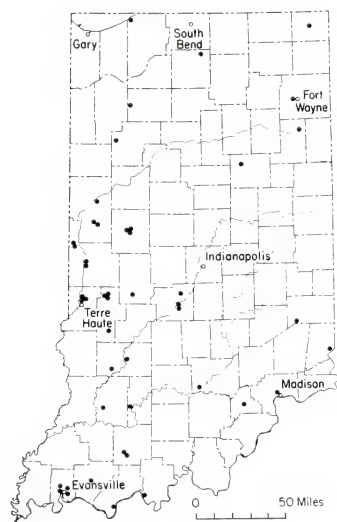


FIGURE 4. *Brick plants of Indiana in 1933 (21).*

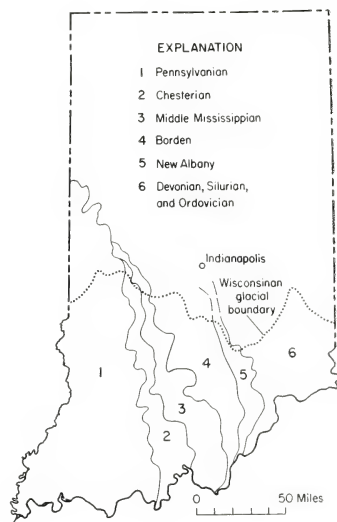


FIGURE 5. *Map showing the distribution of clay materials in Indiana.*

Since 1933, publications on the development of the clay industry have been limited to five directories (1, 6, 12, 14, 16) of the producers and users of clay and shale. Geological aspects of the clays have been explored in one publication (13), and numerous reports have been issued on special aspects of clay mineral research.

Regional Geology of Indiana Clay and Shale Deposits

The clays of Indiana can be divided for discussion into eight types or regions (Fig. 5): 1) Pennsylvanian shales and underclays are near the surface and readily accessible in southwestern Indiana; 2) lying just east of these exposures are shales of the late Mississippian Chesterian Series; 3) in the south-central part of the state, clays lying above the middle

Mississippian limestones comprise a central residual belt, and 4) east of this belt are the shales of the early Mississippian Borden Group; 5) to the east of the Borden outcrops are exposures of the New Albany Shale, and 6) in extreme southeastern Indiana residual clays lie above limestones of Devonian and Silurian age and limestone-shale sequences of Ordovician age. A seventh type of clay material is obtainable at numerous places in northern Indiana, where glacial drift that includes both till and glacial lake sediments can be used as raw material for a ceramic industry. An eighth type of ceramic raw material consists of alluvial clays which are related to present-day streams at various localities throughout the state, and of leached loess found in the southwestern part of Indiana.

In the 19th century and the early part of the 20th, all of these eight types of materials were used by plants producing brick from local raw material. As the 20th century progressed, the brick industry consolidated, not only because of the greater efficiency of larger plants but also because of the superior product that could be manufactured from proper materials. In addition, modern rail and truck transportation provided enlarged market areas. Smaller and less efficient plants were abandoned. Small kilns were replaced by larger ones. Wood as fuel was displaced by coal, which in turn is being replaced by natural gas. Round periodic kilns are giving way to continuously fired tunnel kilns. With growth and automation, very accurate control is necessary over all parts of the brick-making process to produce modern bricks with their wide range of predetermined colors and textures. Clay or shale units that vary greatly in composition or are thin or interbedded with other types of rocks generally have been eliminated as a source of raw material. Unusual rocks or minerals are tolerated only if they can be removed by screening or their deleterious effects minimized by grinding and dispersion. Residual clays overlying various carbonate rocks in southeastern and south-central Indiana and leached alluvial clays throughout the state are not adequate. The thin shales of the Chesterian Series, the carbonaceous New Albany shale, and the commonly variable glacial till have generally been eliminated.

Only shales and siltstones from the Borden Group, a thick sequence of fine-grained Mississippian rocks in south-central and west-central Indiana, and several of the thick shales of Pennsylvanian age found in southwestern Indiana are commonly used in the production of brick. Some brickyards using these materials also consume a lesser amount of the kaolin-rich clay that underlies certain coalbeds in the western part of the state. Kaolin clays increase the firing temperature, widen the firing range, and decrease the firing shrinkage of bricks made from rocks of the Borden Group. For the few firebrick still made in Indiana, underclays are used.

Only one plant, at Munster in northwestern Indiana, now produces brick from glacial materials, but it does have the sizable production capacity of 260,000 bricks per day. The raw material comes from a very clayey, homogeneous till about 22 feet thick.

Types of Bricks

Structural clay products, including common, pressed, and face brick, structural tile, and drain tile, have consumed most of the clay industry's

output. Paving brick was an important product near the turn of the century, but this phase of the brick industry has been almost entirely abandoned. Firebrick for high-temperature ovens, furnaces, and kilns has been made in Indiana for many years. Indiana's fire clays are still well known and marketed in other states, but now only one Indiana company produces firebrick.

The methods used to produce common brick early in the state's history were some of the most simple processes in the manufacture of ceramic ware, and they have been entirely replaced by other techniques. Until the latter part of the 19th century, most building brick in Indiana was made by the soft-mud process. Sandy clays were mixed in a pug mill and either hand molded or forced into sanded molds. The resulting shapes were sun dried or air dried and then burned in kilns, which in their most primitive form were built around the stacked unfired bricks. Sandy clays of the alluvial or loessial types were used for the soft-mud process. The annual production of common brick by most companies operating in 1904 was small, commonly only a few hundred thousand.

In the late 1800's, dry-pressed brick replaced some of the soft-mud variety, but the process was primarily used in the construction of terra cotta and hollow building blocks. Moisture content was kept to the minimum that would allow shaping by compression into molds, and the resulting products were denser and underwent less shrinkage. The raw ware contained only 7 to 10% moisture, but moisture content was 20 to 25% in the soft-mud process.

The use of brick for bearing walls in building construction decreased progressively from shortly after the turn of the century, and pleasing texture and color became relatively more important as brick came to be used mainly for facing. Face brick is produced more satisfactorily by the dry-press and stiff-mud methods than by the soft-mud process.

Since about 1920, face brick produced by the stiff-mud process has been the principal product of the brick industry. A plastic clay mixture is dewatered by vacuum to 12 to 15% moisture content and this is extruded continuously through a die by auger or plunger pressure. Length and width of the brick are controlled by the die, and thickness is established by cutting the extruded bar at desired intervals.

Changes in the Brick Industry

From the 1840's and 1850's, when nearly every community had a brick yard, to the 1890's and early 1900's, when brick as a construction material was at its zenith, the quantity of brick produced grew steadily. At the turn of the century most brick companies were still small, and therefore their number was great. When W. S. Blatchley visited Evansville in 1895, he recorded (2) that 21 brick yards were operating within 2 miles of the county courthouse. In 1905, more than 200 companies in Indiana were producing brick. Of this total, eight used the dry-press process and the rest used the stiff-mud, soft-mud, or hand processes to produce common brick. In 1904, almost 300 million common bricks were produced by these plants, although a high of 315 million had been reached earlier in 1901.

In addition, about 30 million pressed bricks, 50 million vitrified or paving bricks, and lesser amounts of firebrick and ornamental material, such as terra cotta, were produced. The total value of these products for that year was \$2,640,313. Only 34 companies were listed in 1922 in the *Handbook of Indiana Geology* (15), and even though the list is incomplete, the great change in the number of companies involved in brick making, as expressed by the numbers of companies in 1905 and in 1919 when data for the *Handbook* were collected, reflected the change in the building industry in Indiana. The use of common brick was reduced, and wood construction replaced much of the former brick construction. By 1933, when a more complete record of brick plants was made (21), 47 plants produced brick, and most used the stiff-mud process. According to Whitlatch's figures, in 1929 about 112 million common bricks, 130 million face bricks, and 3½ million paving bricks were produced. Fire clay refractories, composed mostly of firebrick, totaled almost 6 million bricks or the equivalent. The total number of bricks was 252 million, valued at \$3,565,823. In 1970 only 12 brick plants remained in the state (Fig. 6), and they produced about 186 million bricks. The value of these bricks was nearly \$8 million. The value of face brick amounted to more than 80% of that total, and the remainder came from sales of common brick.

The brick industry has responded to the modern trend toward the construction of modular homes by the building trades by producing pre-

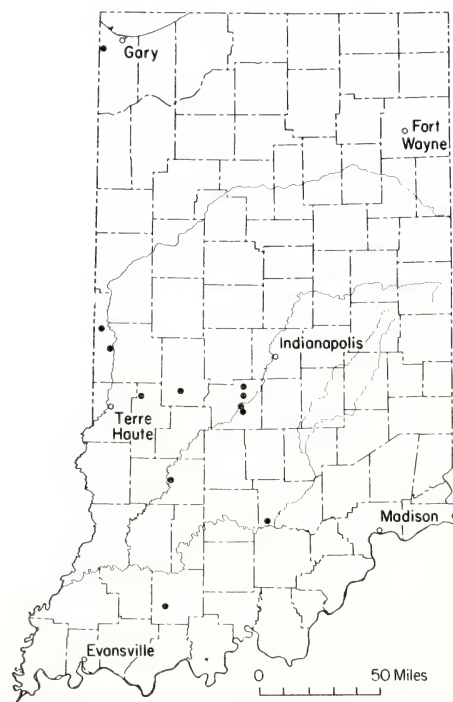


FIGURE 6. Brick plants of Indiana in 1970.

formed brick panels. These panels, which are made with normal or thin brick, are fabricated at the plant, moved by truck to the construction site, and hoisted into place by cranes.

Distribution of Brick

The many small companies that operated in Indiana near the turn of the century probably did not ship their products more than a few miles; however, some larger companies were able to transport their products to destinations many states away. In 1904 the Hoosier Brick Company of New Albany shipped common bricks by rail throughout southern Indiana and Kentucky. During the same year the Cayuga Brick and Coal Company of Vermillion County shipped most of its common brick to Chicago. Dry-pressed brick made at Hobart in Lake County were sold in Philadelphia, Milwaukee, St. Paul, St. Louis, and other cities. The Indiana Paving Brick Company of Brazil began operations in 1891, and by 1904 it was using local shale blended with sand from the shores of Lake Michigan. Paving bricks were shipped from the plant to the larger cities in Indiana and to Cincinnati, Louisville, and many towns in Illinois. Indiana refractory bricks were well known in 1904, and the Burns and Hancock Company of Vermillion County had supplied firebrick to states as far away as Montana, Georgia, and Alabama.

At present most companies find their market within a 200-mile radius of their manufacturing facilities.

Summary

In summary (Fig. 7), by 1900 Indiana's brick industry consisted of more than 200 plants that were distributed broadly throughout the state, that used virtually all types of suitable clay materials, and that produced

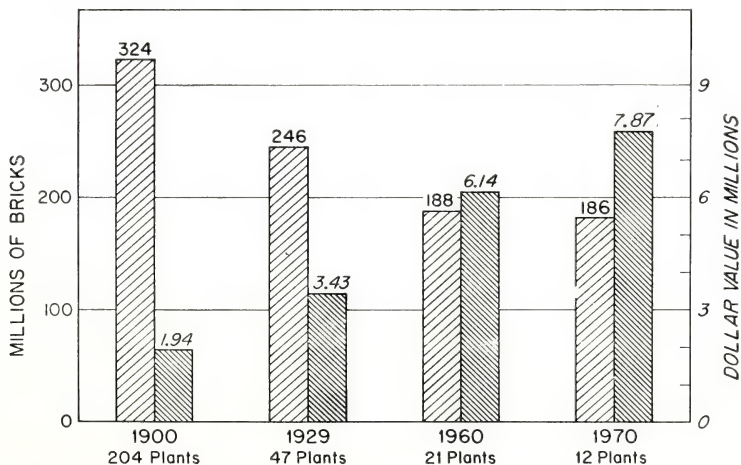


FIGURE 7. *Production and value of brick in Indiana for 1900 (4), 1929 (21), 1960 (5), and 1970.*

more than 300 million brick a year with an annual value of nearly \$2 million, predominantly for common brick. Progressively through the subsequent 70 years the number of plants has decreased to the present 12, the raw materials have been restricted largely to Pennsylvanian shales and underclays and lower Mississippian shales and siltstones, the annual volume has decreased by one-third to slightly less than 200 million brick, and the annual value of the product has grown to nearly \$8 million, partly because of inflation and partly because most of the product is face brick with higher unit value. This is a portrait of a stable industry—one that has lost more ground in relation to the gross state product than is readily apparent from the production figures, but one that is continuing to change with advances in the construction industry and seems likely to continue indefinitely as a significant element in the Indiana economy.

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A Comparison of Public Utility and Governmental Rates and Services Provided Mobile Home Park Residents and Other Residents: A Case Study of Tippecanoe County, Indiana

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Abstract

This study involved a determination of whether services and rates received by mobile home park residents differed from those received by other residents of the county. Differences between these two classes of residents in public utility rates and services were not found in the study area. With the exception of fire protection, mobile home park residents receive governmental services comparable to those received by other residents of the area. The principal variation in rates and services are those between locations inside and outside the municipal boundaries rather than those between residents of mobile home parks and other residents.

Introduction

Mobile homes now account for about 94% of all housing units sold in the U. S. for under \$15,000 and for 2/3 of all housing units costing less than \$25,000. The mobile home business is one of the fastest growing industries in the country today. Because of this growth the number of mobile home parks has increased at a rapid rate.

A literature search revealed no prior investigations comparing quality and costs of public utilities and government services received by mobile home park residents with those received by other residents. Tippecanoe County, Indiana, situated in the west-central part of the state, was selected for such study.

In 1970 a total of 109,378 persons resided in Tippecanoe County, according to the *United States Census of Population*. This was an increase of 20,256 persons over the 1960 census figures. Lafayette, located in the north-central part of the county, is approximately 60 miles northwest of Indianapolis and 120 miles southeast of Chicago. Lafayette had 44,955 persons while West Lafayette contained 19,157 persons, in 1970. Purdue University, with about 25,000 students on the home campus is located at Lafayette. A large number of the university students reside in mobile homes. The size and growth of the population, the presence of Purdue University, and the locational advantages for industrial development, along with the number of mobile home parks, were the reasons for selecting Tippecanoe County, Indiana, for the study. The purpose of the study was to determine whether or not the residents of mobile home parks were subjected to differences in governmental services and public utility rates from the rates and services received by other residents of the study area.

Method

The data were obtained by holding personal interviews with the proprietors and/or managers of the mobile home parks and with the

public utility companies and municipal departments which supply services to the area.

The public utilities were the Public Service Company of Indiana; Tipmont Rural Electric Membership Corporation; General Telephone Company of Indiana; Indiana Gas Company, Incorporated; The City of Lafayette Water Works and Sewage Treatment Department; The West Lafayette Water Company; The City of West Lafayette Sewage Treatment Department; and the Greater Lafayette T.V. Cable Service.

Governmental services included the following: maintenance of streets, curbs, and street lights, police protection, fire protection, and mail delivery.

Findings

It was found that a difference in rates for electrical service exists in Tippecanoe County only in that Public Service, Indiana, Incorporated, has two rate schedules, one for the urban areas and another for rural areas. No rate differentiation is made between mobile home park residents and other residents in the same rate schedule area.

Electrical service is made available to the mobile home park as freely as to any other residential development in the county. Public Service Indiana has encountered no major problems other than that of congestion of lines with the other public utilities during the period of construction.

Indiana Gas Company, Incorporated, sets no rate differences among its various types of customers. One distinction is made in that mobile home park residents are placed in the same class as customers who rent, buy on contract, or live in an apartment. All of these residents are required to pay an \$18.00 deposit before the gas company will provide service to the unit.

Gas service is made available to any developer who wishes to make a deposit equal to the cost of construction in case the gas company does not feel it is economically sound for it to pay the initial cost. The Indiana Gas Company does not face any major problems in bringing service to the mobile home park resident other than that faced by other utilities, the congestion of lines during the construction period.

A comparison of rates for water service in the mobile home parks with other residents of the county revealed no difference between the two; however, service was not as freely available to the mobile home parks, primarily because it is not economically sound for the mobile home park developer to pay for the construction of water lines when he can drill his own well at a much lower cost. When service is extended beyond the city limits of Lafayette the Board of Works decides who will absorb the cost of construction for the water and sewage lines. In West Lafayette the water company will not provide service to a mobile home park unless the developer makes a deposit equal to the cost of construction which is estimated by the West Lafayette Water Company prior to the construction. It should be noted that the majority

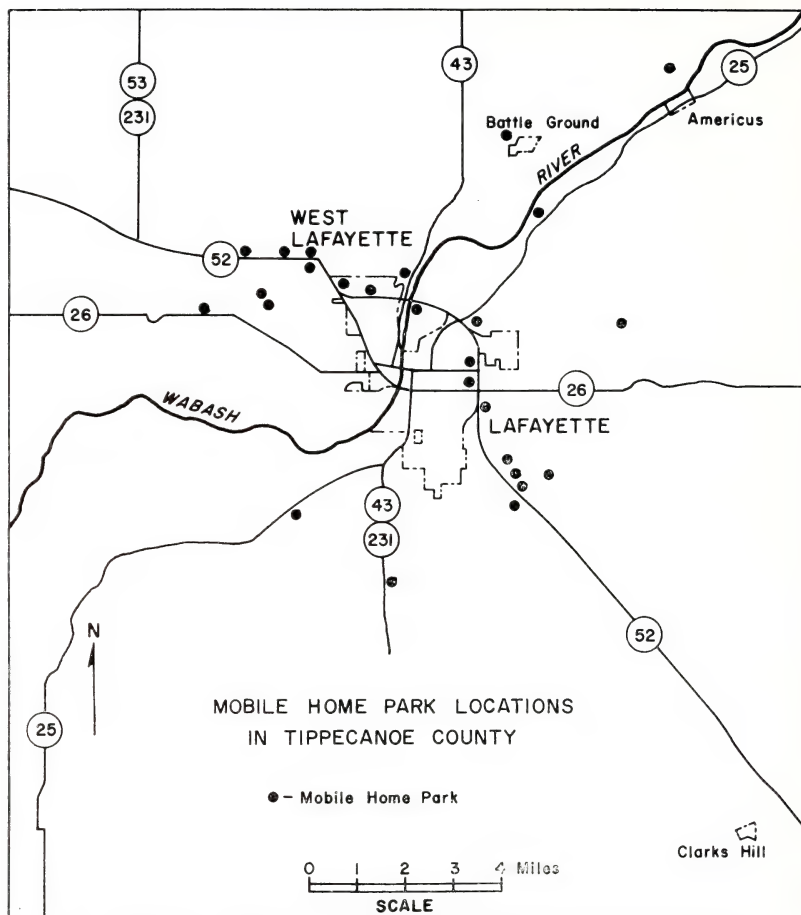


FIGURE 1. *Mobile home park locations in Tippecanoe County, Indiana*

of the mobile home parks are located just outside the municipal boundaries (Fig. 1).

A developer may construct his own sewage lines and connect them to the city's lines without having city water service if he is able to obtain a permit by the city. However, it is more economical for the developer to install septic tanks than to pay the cost of construction of city sewage lines. No park in the study had city sewage without city water service.

General Telephone Company of Indiana assigns its rates by zones with Lafayette as the base area. No distinction is made between mobile home park residents and other residents. A mobile home park resident will, as will any other resident, pay a rate dependent on the zone within which he is located.

Telephone service is available to all of the mobile home parks included in the study. The major problem faced by General Telephone is the strain placed on the central office as service is extended to a new area.

The Greater Lafayette TV Cable Service Company does not distinguish between mobile home park residents and other residents when establishing rates. However, the parks are treated as apartment dwellings in installation and rate determination. This classification often produces a rate which is lower than that for other residents. Only six mobile home parks have cable T.V. service. The major problem is again the congestion of lines.

In the area of fire protection the mobile home parks, because of being located mostly outside the municipal boundaries, generally do not receive the same level of service as do the residents of Lafayette and West Lafayette. Parks located outside the municipal boundaries must rely upon volunteer departments. Such units require an average of approximately 25 min. to respond to calls, too long a time for the department to be effective in extinguishing a mobile home fire. As a result of being located relatively close to fire stations, municipality residents can be reached more quickly by the full-time fire fighting units.

Police protection of mobile home parks is comparable to that of the other residents of the county. The mobile home parks located outside the municipal boundaries are protected by the Sheriff's Office and the Indiana State Police. The parks within the municipal boundaries are protected by the municipal police departments.

Maintenance of streets, curbs, and street lights alongside the mobile home park is also at the same level of service as that of any other area within the county. The streets, curbs, and street lights within the parks are the responsibility of the park owner.

The United States Post Office Department furnishes the same service to the residents of the mobile home parks as to the other residents, the postman going to each unit in the park.

In summary, it can be stated that there are no differences between the mobile home park residents and other residents in rates of the public utilities, and that service is available to any park if the developer wishes to make the investment when the public utility does not feel it is economically sound for it to do so.

With the exception of fire protection, in the area of governmental services the mobile home park resident receives comparable service to that received by other residents who are similarly located. In general it can be concluded that the principal variations in rates and service are those between locations inside and outside the municipal boundaries rather than those between residents of mobile home parks and other residents.

Heavy Water—A Natural Tracer

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Abstract

All naturally occurring water contains a small percentage of isotopically heavy water molecules. The processes of vaporization and condensation affect the concentration of the heavier water molecules. The changes in concentration of heavy water molecules have been used to study the origin of winter storms, to determine evaporation rates from lakes, and in studies of water movement.

Introduction

Three common heavy water molecules occurring in nature are HDO, HTO, and H_2O^{18} . HDO and H_2O^{18} are stable (non-radioactive) isotopic forms of water and comprise 0.015% and 0.20%, respectively, of naturally occurring water. HTO is radioactive and emits low energy beta radiation. A small amount of HTO occurs naturally, but the use of nuclear devices has increased the amount of HTO in the hydrologic cycle.

The "heavy water" associated with nuclear projects, D_2O , is not important in natural water supplies as it tends to break down and cause the formation of HDO.

Scientists usually work with the natural or bomb-introduced concentrations of heavy water. But, sometimes it is possible to utilize small amounts of artificially concentrated heavy water for a specific study. The concentrations of HDO or H_2O^{18} in a water sample are usually measured with a mass spectrometer. A liquid scintillation counter must be used to measure HTO.

The use of isotopically heavy water as a tracer of water movement is best explained through a series of examples. First, two concepts must be explained.

The vapor pressure of heavy water is slightly less than the vapor pressure of H_2O^{16} at the same temperature. This will cause a slight fractionation to occur whenever the processes of evaporation or condensation takes place. During evaporation the heavy molecules tend to become concentrated in the liquid phase while the vapor phase has a higher percentage of light molecules. During condensation the heavy molecules will tend to condense out more rapidly causing the first condensate to be enriched with heavy molecules and the remaining vapor to have a greater percentage of light molecules.

The heavy water content of a sample is often given as the per cent deviation of the heavy isotope content of the sample relative to the heavy isotope content of average ocean water. A sample of precipitation with a HDO content reported as -7% contains 7% less HDO

molecules than ocean water, namely a reference water (SMOW, standard mean ocean water, as recommended by Craig (1)). Precipitation is usually isotopically lighter than ocean water.

Examples

The change in the heavy isotope content of a body of water can be used to determine the evaporative loss from that body of water. The water entering a large lake has an average HDO content of -8.3% and the discharge from the lake has a HDO content of -7.6% (Fig. 1). These measurements indicate that fractionation during evaporation caused an enrichment of the heavy isotope content of the lake water. A knowledge of the amount of increase of HDO in the lake and the vapor pressures of H_2O and HDO allows one to calculate that 15% of the original volume of water has evaporated.

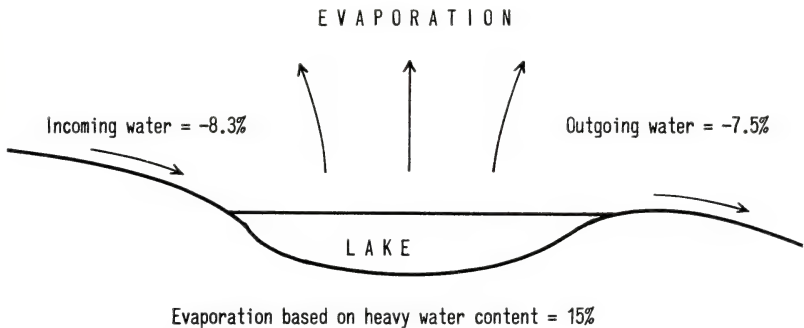


FIGURE 1. Diagram of heavy water—evaporation relationships of a lake. The amount of evaporation from a lake can be determined by comparing the HDO content of the incoming water with the HDO content of the outgoing water. Diagram after Friedman et al (1964).

On a larger scale, we can follow the changes in the HDO content of the moisture in a storm system (Fig. 2). The HDO (and H_2O^{18} content of precipitation is controlled by the amount of condensation and the temperatures at which this condensation takes place. The moisture evaporating from the ocean will be isotopically lighter than the ocean water. As the processes of evaporation and condensation are opposite, the first condensate from the air mass will have about the same heavy isotope content as the original ocean water. As the air mass moves farther inland it will become depleted in heavy molecules and the resulting precipitation will be isotopically lighter. Snow is usually isotopically lighter than rain and high altitude precipitation is usually lighter than low altitude precipitation.

In California, isotope measurements have been used as an indication that during a winter of heavy snowfall there may have been a change in circulation patterns not detected by the conventional meteorologist. An extensive series of HDO or H_2O^{18} measurements should help the meteorologists obtain some quantitative information on the processes

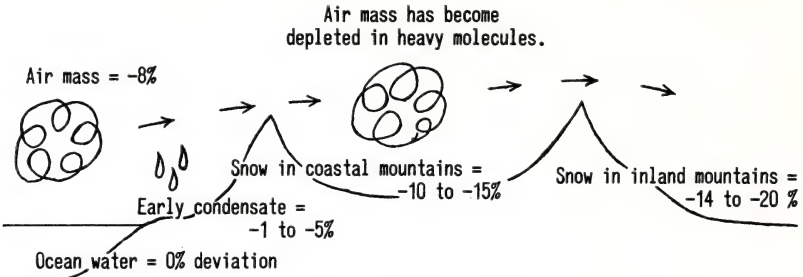


FIGURE 2. Diagram of the variations in the HDO content of the precipitation as an air mass moves inland. Preferential condensation of the heavier molecules causes the moisture in the air mass to become isotopically lighter as it travels farther inland.

occurring in the atmosphere. The main problem is a lack of sufficient available data on the heavy isotope content of precipitation.

The variation in the heavy isotope content of precipitation may be used in some hydrologic studies. Perhaps a city near a mountain range is dependent upon ground water for a public water supply and the city would like to determine the recharge area of the aquifer they are using (Fig. 3). Basic information could be obtained by measuring the heavy isotope content of the ground water and comparing it to the heavy isotope content of precipitation in various areas. If the well water was isotopically light, it could be assumed that the recharge area was high in the mountains.

The uses of the isotopically heavy forms of water are not limited to those just mentioned. HDO has been used in snowmelt studies, to

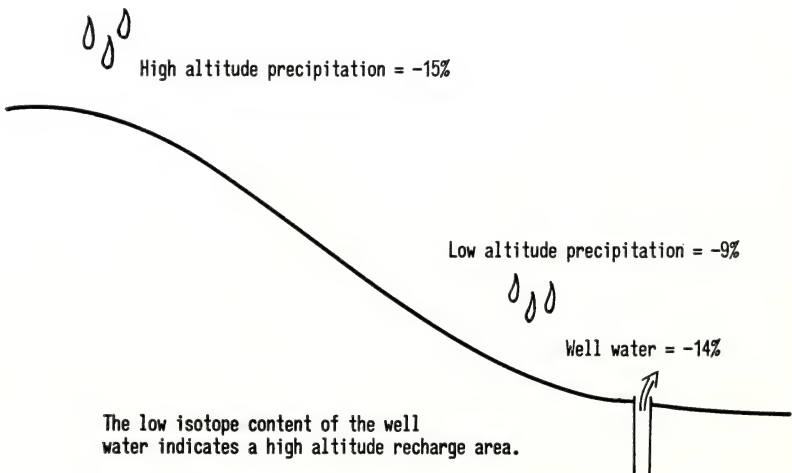


FIGURE 3. Diagram of how the HDO content of water of unknown origin can be used to indicate which of several areas is the actual source of the water.

help date layers of ice in glaciers, to trace the movement of soil moisture, and in biological studies. The list of references that follows expands on several of the applications. Friedman *et al.* reviewed much of the work done with HDO (3). Dansgaard reviewed some of the work done with H_2O^{18} (2).

There are several advantages to using heavy water as a tracer of water movement. Water is actually being used to trace water, so there is little problem with the selective absorption of an introduced tracer. HDO and H_2O^{18} are naturally occurring and are not radioactive so people will not be upset by their use. The slight fractionation upon evaporation and condensation allows a quantitative measurement of many hydrological and meteorological processes.

There is one disadvantage. The cost of sample analysis (currently about \$50 per sample) is high. This has restricted the accumulation of large amounts of data and background knowledge about HDO and H_2O^{18} in the hydrologic cycle.

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A Preliminary Investigation of Sludge Refuse from Indiana Coal Mines¹

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Abstract

Seven samples of fine refuse (sludge) were collected from two coal mine refuse pits located in Greene and Sullivan Counties. Particle size distribution was determined. Proximate and ultimate analyses of the sludge refuse were determined and compared to a "whole coal." The data indicated that the British Thermal Unit content of sludge refuse was about 75 per cent of that of the "whole coal" on the moisture-free basis, but was almost similar on the moisture-ash free basis. Based on chemical parameters, sludge refuse should be considered a natural resource for future fuel use and should be reclaimed.

Before coal can be used by industry, it must undergo a preparation process at the mine site. This process consists of crushing, washing, de-watering, and sizing of the coal. During the washing cycle, refuse (pyrite, shale, clay, calcite, rock, high-gravity coal, etc.) is separated from the coal.

Coal preparation produces two kinds of refuse: 1) a coarse particle refuse ("gob") that may be several inches in diameter; and 2) a fine particle refuse ("sludge" or "tailings") that may be 1/8 inch or less in diameter. For brevity the terms "gob" and sludge are used in this paper. Sludge contains larger percentages of coal than does "gob." Total refuse can, periodically, account for as much as 45% of the total daily raw coal (unprepared coal) production. Generally, the average percentage for sludge is 3-7% and for gob 9-15% of total daily raw coal production.

Within the 22 years preceding 1970, Indiana mines produced 370,022,689 tons of salable coal (M. B. Fox, personal communication). Because almost all of this coal underwent the preparation process, the average percentages (sludge—5% and "gob" 12%) may be used to calculate the amount of refuse produced. Calculations indicate that during the 22-year period 136,857,706 tons of refuse were produced of which 40,326,165 tons were sludge.

Sludge produced from the preparation of the following coal members is considered in this paper: 1) Survant Coal (IV), 2) Springfield Coal (V), 3) Hymera Coal (VI), and 4) Danville Coal (VII) (2).

Greene County

The Greene County sludge pit covered an area of 80-90 acres from which Samples #4, 6 and 5 were taken (in that order). The interval between samples was 3,000 feet. Sample #4 was 1,000 feet from the point of sludge entry into the pit. For observational purposes, additional

¹Publication authorized by the State Geologist, Department of Natural Resources, Indiana Geological Survey.

samples were taken at 100 feet intervals between Sample Sites #5 and 6. Between these sites there was an apparent change to smaller particle size and to a "dirty gray" color.

Sullivan County

The Sullivan County sludge pit covered a 70-acre area from which Samples #13, 14, 11, and 12 were collected (in that order). Sample #13 was the nearest to the point of sludge entry (about 400 feet). The interval between samples was about 425 feet.

Methods

Seven sludge samples were collected from two sludge storage areas (pits) of mines located in Greene and Sullivan Counties. Before sampling, about 10 inches of sludge surface was removed and then the sample was taken with the use of a post hole digger to a depth of 4 feet. The sample filled a 5 gallon can.

Sample preparation and subsequent chemical analysis followed the procedures and specifications of ASTM-D-271-58 (1). After collection, the samples of sludge refuse were air dried so that their moisture content was in equilibrium with that in the atmosphere. Each sample was thoroughly mixed and then split into four equal parts. Two of the parts (making up 50% of the original sample) were mixed back together to form the sample for the particle size determination. Of the remaining two quarters, one was put into storage for future work and the other was used for chemical analysis.

The chemical analysis consisted of determining moisture, ash, volatile, fixed carbon, total carbon, nitrogen, hydrogen, and sulfur along with the BTU and the CO_2 . These data for sludge are compared with the data from "raw coal." The Greene County sludge is compared with Coal V while the Sullivan County sludge is compared with Coal VI.

The screening of the air-dried sample was accomplished with the use of U.S. Standard sieves and a Tyler Ro-Tap sieve shaker. The sieves had square opening sizes of 8, 16, 32, 100, and 200 mesh.

Results and Discussion

On the moisture-free basis the data indicate that sludge from the Greene and Sullivan County pits is inferior to their respective comparative coals (Tables 1 and 2). However, if data on the moisture-ash free basis are considered, it is found that the differences are reduced and that similarity of the materials is emphasized (Tables 1 and 2). Samples #13, 14, and 11 of the Sullivan County pit exhibit the idealistic pattern; that is, higher ash and lower BTU nearer the point of sludge entry into the pit progressing to lower ash and higher BTU farther away (Table 2). Sample #12 appears to be the maverick in the group with its high ash and low BTU; but the data in the particle size histogram of Figure 1 indicates that Sample #12 had a high percentage of fine particles (other data determined on particle size but not presented in this paper indicated that higher ash is located in the finer size).

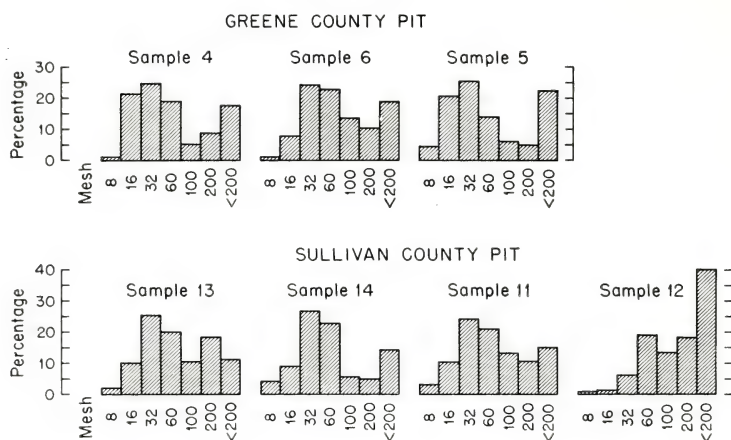


FIGURE 1. Histogram showing particle size percentages.

Sample #6, of the Greene County pit, exhibited the same tendencies as Sample #12 except that its (Sample #6) sulfur and CO_2 were very high. These high values were brought about by a secondary washing from run off water of a nearby 100-160 acre farm. The run

TABLE 1. Proximate and ultimate analysis, Greene County Pit.

	Coal	“Sludge”		
Sample	V	4	6	5
		Moisture Free		
Ash	10.2	29.1	44.7	22.2
Volatile	40.9	29.0	27.4	31.7
Fixed Carbon	48.8	41.8	27.9	46.0
Carbon	71.1	54.3	41.3	61.3
Hydrogen	4.93	3.52	2.91	4.14
Nitrogen	1.54	0.85	0.70	1.07
Sulfur	3.9	3.29	6.97	3.46
BTU	12,670	9,560	7,130	10,880
CO ₂	1.33 ¹	0.08	2.98	0.12
		Moisture and Ash Free		
Volatile	45.5	41.0	49.6	40.8
Fixed Carbon	54.3	59.0	50.4	59.2
Carbon	79.2	76.6	74.7	78.9
Hydrogen	5.49	4.96	5.26	5.32
Nitrogen	1.71	1.20	1.27	1.38
Sulfur	4.34	4.64	12.6	4.45
BTU	14,110	13,480	12,900	14,000
CO ₂		0.11	5.39	0.16

¹sample only

off water entered the pit just above Sample #6 site resulting with the separation of the lighter particles of coal from the heavier particles of pyrite and calcite.

Because moisture is an erratic variable (on a daily basis) the data for it were not included in the tables; but the moisture percentage ranged from 18.8 to 37% with an average of 25.6% on the "as received" basis.

TABLE 2. *Proximate and Ultimate Analysis, Sullivan County Pit.*

	Coal	“Sludge”			
Sample	VI	13	14	11	12
	Moisture Free				
Ash	13.4	26.6	19.3	18.7	34.3
Volatile	39.6	32.1	31.5	34.6	25.7
Fixed Carbon	47.0	41.3	49.2	46.8	40.0
Carbon	68.6	57.4	61.7	63.6	52.1
Hydrogen	4.76	2.85	4.12	4.50	3.27
Nitrogen	1.55	1.19	1.30	1.35	0.94
Sulfur	3.80	3.09	2.62	2.23	1.81
BTU	12,040	9,950	10,960	11,310	8,950
CO ₂	0.16 ¹	0.37	0.13	0.13	0.16
	Moisture and Ash Free				
Volatile	45.7	43.7	39.0	42.5	39.1
Fixed Carbon	54.3	56.3	61.0	57.5	60.9
Carbon	79.2	78.2	80.0	78.2	79.3
Hydrogen	5.50	3.89	5.11	5.53	4.98
Nitrogen	1.87	1.62	1.61	1.66	1.43
Sulfur	4.39	4.22	3.25	2.75	2.76
BTU	13,900	13,550	13,580	13,910	13,620
CO ₂	0.18 ¹	0.15	0.16	0.16	0.25

¹Sample only

Conclusion

The data bore out some of the visual observations. When considering the BTU on the moisture-free basis, sludge refuse contains about 75% of that of the compared coal (Table 2). If the ash can be eliminated then sludge refuse and coal are very similar, as indicated by the moisture-ash free basis data. Because of the similarity of the chemical parameters, and because of the great quantities available (over 40 million tons), sludge refuse should not be wasted. It should be considered a fuel and should be reclaimed for fuel purposes.

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The Distribution of Slopes in Indiana

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Abstract

Slope maps of Indiana have been prepared from the 2 percent sample of the United States Department of Agriculture Conservation Needs Inventory. The Syngraphic Mapping Technique (a form of computer cartography) was used to generate maps of average slope, slopes between 0 and 2 per cent, and slopes 35 per cent and greater.

Introduction

The objective of this study was to develop a quantified slope map which would supplement and extend the work of Malott (2) on the regional geomorphology of Indiana. A subsidiary objective of the research was to provide a slope map, sufficiently detailed, to aid in the development of more precise morphometric measures to be used in studies of watersheds in Indiana.

The classical study classifying and describing the physiographic regions of Indiana was published by Mallott in 1922 (2). More recent summaries of the State's physiography are to be found in papers by Schneider (4) and Wayne (6). A quantitative approach to the physiography of the state is taken by Lewis (1) who used principal components analysis as a method of regionalizing 13 morphometric variables. His data were collected from a random sample of 60 fourth order drainage basins. The writers believe that Lewis is justified in concluding that his approach provides a more objective view of Indiana physiography. However, any examination of the benefits of objectivity as yielded by principal components analysis must be balanced by the fact that the physiographic regions, so produced, are rudimentary and not particularly informative.

In the geographic literature there are many reports of research dealing with state and regional maps of slope, though none of these studies use the mapping techniques and data sources of this paper. Good summaries of slope research are to be found in Miller and Summerson (3), and Zakrzewska (7).

Conservation Needs Inventory

It was recognized in the mid-1950's that changes in land-use patterns with their associated problems were taking place rapidly enough to necessitate the development of a data bank which could be used to monitor these changes. The response to the situation was the establishment, in 1957, of the Conservation Needs Inventory (referred to hereafter as the C.N.I.). The major objective of this Inventory was to provide the U.S. Government with up-to-date and detailed information

on the rural landscape. The scope of the data is indicated by the following variables which are included in the inventory: land ownership, soil type, slope, erosion, land use and treatment needs. The U.S. Department of Agriculture wanted the inventory, for the entire country, to be completed within 3 years of the starting date in 1957. To achieve these objectives, Taylor (5) designed a stratified random sample, which used the quarter section (160 acres) as its standard sampling unit; a sampling rate of 2% was established that required the selection of 3 quarter sections per township. The Statistical Laboratory of Iowa State University in Ames, Iowa, is responsible for maintaining and periodically updating the Inventory which is stored on magnetic tape.

Analysis of the Data

The first task of this research was to change the order of the C.N.I. variables from a sequence based on soil types to one based on counties and townships. This strategy put the data into a locational form eminently suitable for computer mapping.

With the data in the new sequence it was possible to compute the average slope value for a township by using the following formula:

$$Y_i = \frac{\sum_{j=1}^7 P_j \left(\sum_{k=1}^N q_k \right)}{S} \times 100$$

where, Y_i = average slope for the i^{th} township

N = number of soil mapping units in the i^{th} township containing at least one acre of slope class j

P_j = midpoint value of slope class j (%)

q_k = acreage of the k^{th} soil mapping unit

S = sample acreage of the i^{th} township.

The outer summation refers to the seven slope classes adopted by the C.N.I. for Indiana; these are 0-2; 2-6; 6-12; 12-18; 18-25; 25-35, and greater than 35% slope.

Computerized maps of the slope classes were then generated by using the Synagraphic Mapping Technique (SYMAP). The geographic center of the 1,270 townships used in the Inventory of Indiana served as the data points for the maps.

Slope Maps of Indiana

Four maps were selected for illustrating the variation of slope in Indiana. Included were two average slope maps, and maps of 0-2% slope and 35% and greater slope.

Average Slope Map 1

This map (Fig. 1) uses the slope class intervals adopted by the Conservation Needs Inventory for Indiana (these values vary from state to state). The feature that clearly emerges from the map is the division

of the state into two parts about a line drawn approximately east-west through Indianapolis. The slopes of the southern half of the state clearly reflect the physiographic divisions established by Malott (2). The Wabash and Scottsburg Lowlands are readily identified as are the higher average slopes of the Crawford, Norman, and Dearborn Uplands. The Mitchell Plain between the Crawford and Norman Uplands can be distinguished even though the pattern of low slope is broken into discontinuous units. The northern half of the map is dominated by 1-6% slopes reflecting the glacial modification of the landscape north of Indianapolis.

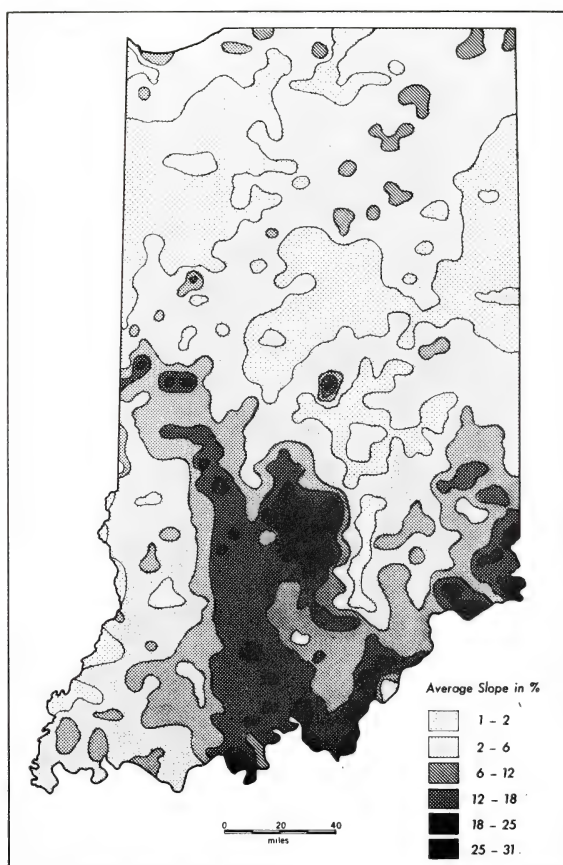


FIGURE 1. *The average slope map 1.*

The 0-2% Slope Map

The value of this map (Fig. 2) to the interpretation of Indiana physiography is the emphasis it gives to the occurrence of low slopes, which are indicated by the darker shadings on the map. For example,

the Calumet and Maumee Lacustrine Lowlands are clearly differentiated as well as the more extensive Tipton Till Plain to the south. The lowlands and floodplains associated with the Kankakee, Wabash and White Rivers are well defined areas of low slope. Other features that can be identified are the Valparaiso Moraine and the Steuben Morainal Lake Area.

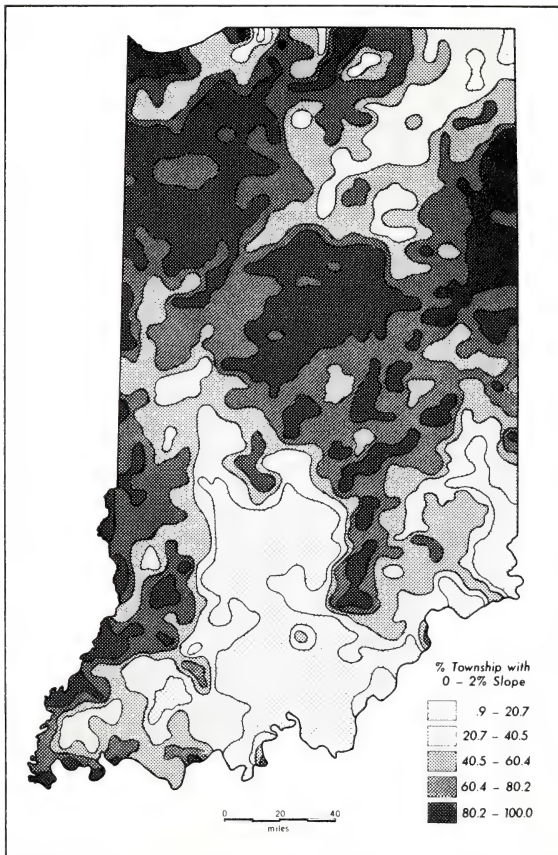


FIGURE 2. The map of slope in Indiana based on the percentage of a township with 0-2% slopes.

Average Slope Map 2

The class intervals for this map (Fig. 3) are changed from Figure 1 in that each interval represents an equal range of slope. It is apparent that the lowest class interval does not discriminate, very effectively, the slope variation in the northern half of the state. The concentration of high slopes in the southern uplands of Indiana is more clearly em-

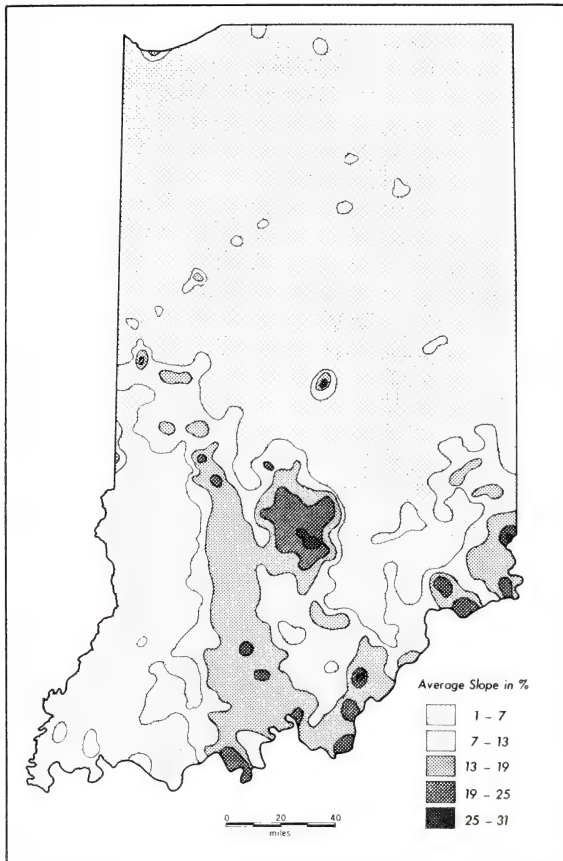


FIGURE 3. *The average slope map 2.*

phasized than on the previous map and, again, the Malott physiographic divisions are reaffirmed in this area.

The 35% and Greater Slope Map

The high slopes, on this map (Fig. 4), are spatially clustered into two groups. One group is associated with the bluffs of the Ohio River, while the other is a cluster extending NW-SE across the southwestern quadrant of the state. This trend line appears to reflect the occurrence of particularly high slopes in both the Norman and Crawford Uplands. An area of high slope in the central portion of the map reveals a problem of widely scattered data points in relation to the SYMAP algorithm. The data of one township with a high slope value located on the bluffs of the White River are expanded to influence a much larger area on the map than exists in reality. This difficulty can be overcome when the sample is areally dense enough to prevent the present situation of an isolated anomaly from arising.

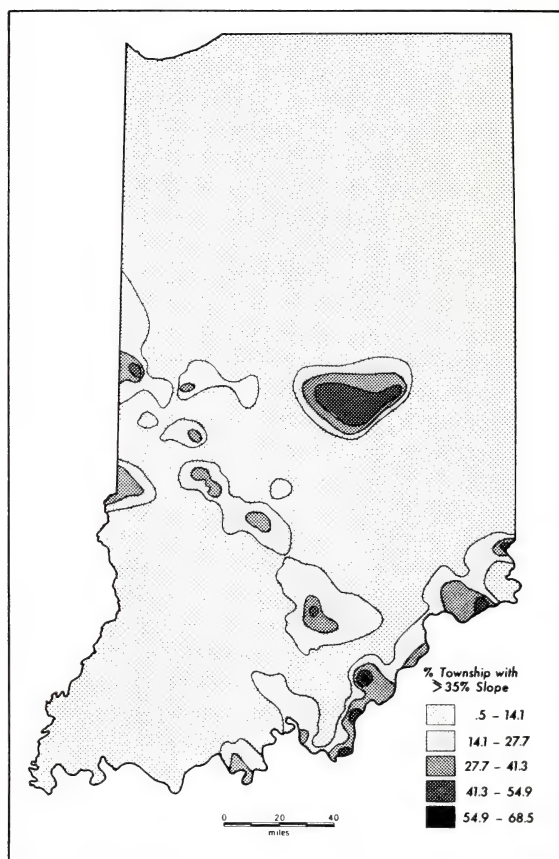


FIGURE 4. *The map of slope in Indiana based on the percentage of a township with > 35% slopes.*

Conclusions

The value of the C.N.I. to the physical geographer who is concerned with slope research has been demonstrated. The maps produced here give insight into the variation of landforms of Indiana for they show that physiographic regions are not uniform spatial units but, in fact, units possessing internal variations.

Acknowledgment

We thank Mr. J. M. Hollingsworth, Staff Cartographer, Department of Geography, for his contributions in the preparation of the maps.

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MICROBIOLOGY AND MOLECULAR BIOLOGY

Chairman: ROBERT F. RAMALEY, Department of Microbiology,
Indiana University, Bloomington 47401

MORRIS POLLARD, Department of Microbiology, University of
Notre Dame, Notre Dame, Indiana 46556, was elected chairman
for 1972

Abstracts

Comparative Growth Kinetics of Microbial Bio-Oxidation. ROBERT H. L. HOWE, Eli Lilly and Company Tippecanoe Laboratories, Lafayette, Indiana 47902.—The comparative growth kinetics of microbial bio-oxidation was discussed. A number of mathematical explanations were devised and compared. For a substrate of high concentration, the kinetics of both batch and continuous systems were presented. Illustrations were given for signifying the difference of the first order kinetics and those of higher orders.

A Survey of Pollution Levels in the Bean Blossom Watershed (Lake Lemon) and in the Salt Creek Watershed (Lake Monroe)—Brown and Monroe Counties, Indiana. JAMES BUTLER, DAVID DOCAUER, MARK DOWNING, MICHAEL DULIN, KENT FISCHVOGT, WILLIAM GARDINER, JAMES HOLLOWAY, JOHN JACOBY, JERRY NEFF and LINDA SCHELL. Indiana University Undergraduate Honors Program and Department of Microbiology, Bloomington, Indiana 47401.—One hundred and twenty-two samples were taken from various areas within the Lake Monroe watershed by the H-300 Environmental Protection and Planning class during the spring of 1971 and analyzed for presumptive coliform numbers by the most probable number tube dilution method. Selected samples were also analyzed by the confirmed and completed tests. One hundred and thirty samples were similarly analyzed from the Lake Lemon and Bean Blossom Creek watershed. The detailed maps and results of the study are on file at the Indiana State Board of Health. Areas within the Lake Monroe area that showed unusually high coliform levels were: 1) downstream from the City of Nashville sewage treatment plant on the north fork of Salt Creek; 2) areas adjacent to boat launching ramps; 3) Moore's Creek and Ramp Creek; 4) bottom samples from the Fairfax marina; and 5) a number of isolated areas in the upper watershed. The swimming beaches and the center of the lake showed a negligible coliform level. In contrast to the low levels of coliform in the relatively undeveloped Lake Monroe watershed, the Lake Lemon and Bean Blossom watershed showed 100 presumptive coliform or greater per milliliter in more than 20 of the samples taken. These high coliform levels in a similar and adjacent unprotected watershed point out the necessity for continued environmental protection of Lake Monroe watershed by the Indiana State Board of Health.

The Use of Pollution Surveys as Aids in Environmental Protection and Planning. ROBERT F. RAMALEY, Department of Microbiology, Indiana University, Bloomington, Indiana 47401.—During the past 3 years

a large number of students both in courses and during independent study have conducted pollution surveys from this laboratory (*e.g.*, the H-300 survey of Lake Monroe) upon the request of conservation and environmental groups and city and county planners. In these surveys we have tried a number of different procedures and survey methods, some of which may have utility especially to biology classes in Indiana's primary and secondary schools. It is to be anticipated that there will be an increasing number of public pollution surveys conducted by classes in Indiana schools and universities, and, in order for these surveys to have their desired effect, the following suggestions are offered: 1) *The use of Difco Endo LES agar rather than the presumptive coliform tube test.* It is a better teaching aid and is much more selective than other liquid or solid media. It is so highly selective for coliform that schools without sterilizing equipment for sterilizing media will be able to use it without any problem provided they make it up fresh before each use and protect it from light. We have found that 0.1 milliliter of water sample spread over the surface of the agar is usually sufficient for the determination of the number of coliform in most samples. However, a series of samples should be prepared if millipore filters are used to sample larger volumes since high bacterial growth may obscure the identification of coliform colonies, giving a false negative test. 2) *Coordination with local and state regulatory agencies* both in the planning and in the interpretation of the results. There is increasing evidence that the number of fecal coliforms is more closely related to the level of sewage pollution, and, although this test is not unusually difficult, it is best that some duplicate samples be done by experienced bacteriological laboratories such as the Indiana State Board of Health. 3) *Cooperation with local city and county planners* so that survey information will be as useful as possible in deciding the future extent and location of development as well as simply pointing out present problems.

NOTE

The Impact of Water Pollution on Recreational Areas in Delaware County. STANTON C. BURT, Department of Microbiology, Indiana University, Bloomington, Indiana 47401.—The contamination of lakes, rivers, and their tributaries with fecal material often results in the conversion of recreational areas into health hazards. This is an extensive problem which can be combated most easily by the application of state laws through local agencies.

Methods and Materials

The procedures followed for the determination of coliform group are those in *Standard Methods for the Examination of Water and Wastewater* published by the American Public Health Association. The "presumptive" and "confirmed" tests were performed before the application of the MPN chart.

Results

During the summer of 1971, Muncie Creek, which empties into White River within McCulloch Park, had an average coliform count of 130,000

organisms per 100 milliliters as it flowed through the park. The Muncie Mall and Marhoefer Packing Company outflows were investigated as possible contributors to this situation. Two-thirds of the Muncie Mall samples exceeded the million organisms per 100 ml. The Marhoefer samples varied from a low of 240,000 to a high of 1,100,000 organisms per 100 ml depending upon the time of day they were taken. The Regulation SPC 1R-2, Water Quality Standards For Waters of Indiana, states that "Coliform group (is) not to exceed 5,000 per 100 ml as a monthly-average value (either MPN or MF count)." This places the Muncie Mall and Marhoefer Packing Company as much as 220 times the violation figure at times.

The Prairie Creek Reservoir swimming facility was found to have an MPN of 15,000 per 100 ml. A creek, which drains a feedlot $1\frac{1}{2}$ miles upstream, empties approximately one block from the swimming site. This sloping feedlot is located near the corner of 700E and 500S and multiplies the coliform group level of the stream 30 times.

Data cannot relay the offensiveness of the Nebo Bridge outflow which dribbles toilet paper down the bank and into White River. Although residential outflows such as this one are small they are often the most potent as all samples having an MPN of greater than 1,100,000 per 100 ml would indicate.

The local Stream Pollution Control Board held hearings for the Muncie Mall and Marhoefer Packing Company reaching solutions agreed to by both parties. The Nebo Bridge outflow and the feedlot were outside the sanitary district's jurisdiction but will fall under county health laws. There are 123 outflows remaining to be investigated.

The Effects of Glucose, Acetate, and Malonate Upon Fatty Acid Distribution in *Aspergillus niger*.

DAVID L. PETERSON and ALICE S. BENNETT,

Department of Biology,

Ball State University, Muncie, Indiana 47306

Abstract

Submerged cultures of *Aspergillus niger* were grown in media containing different concentrations of glucose, sodium acetate, or sodium malonate. Varying concentrations of glucose with or without added acetate did not alter the distribution of fatty acids produced by 120 hour cultures.

Increasing the concentrations of sodium acetate in the 5 per cent glucose medium caused the percentage of saturated fatty acids to increase from 20 per cent to 68 per cent. However, aerated stir cultures containing 2.5 per cent acetate produced the normal amount of unsaturated fatty acids with linolenic acid representing the largest component. In the control cultures linoleic is the major unsaturated fatty acid.

The high acetate cultures also differed from the control in that their growth was markedly inhibited, and the sharp decrease in pH which is characteristic of *Aspergillus* cultures was not observed.

Similar but not as pronounced effects were observed when sodium malonate was added to the 5 per cent glucose medium.

Introduction

Research in recent years has indicated that there are at least two pathways for the biosynthesis of fatty acids. The acetyl-coenzyme A pathway has been observed in intact mitochondria isolated from plants (1, 2, 3, 8, 13, 19, 21) and mammals (6, 7, 18). The malonyl-coenzyme A pathway, which occurs outside of the mitochondria, has been identified in many plant and animal tissues (20, 21). In soybeans (9), isolated plant leaves (10), and yeast (5) the long chain fatty saturated fatty acids are subsequently converted to unsaturated fatty acids by an oxygen dependent enzyme system.

Members of the Class Ascomycetes, with fatty acid compositions similar to those found in higher plants (15), have been used to study the biosynthesis of fatty acids in plants and plant-like organisms (4, 11, 17). Matto (12) reported that fatty acid synthesis in *Aspergillus niger* was reduced but not completely inhibited when avidin, a biotin inhibitor, was added to the medium, suggesting that fatty acids are synthesized by a pathway other than that using malonyl-coenzyme A. If the alternate pathway utilizes acetate, it should be possible to stimulate fatty acid synthesis by this pathway by increasing the amounts of acetate in the presence of a carbohydrate source in the medium. However, Romano and Kornberg (14) have reported that cultures of *Aspergillus nidulans* grown in a medium containing 100 mM of sodium acetate and 100 mM of glucose were markedly inhibited in growth and their ability to incorporate hexoses. The fatty acid distribution of the inhibited cultures was not reported.

In this study the effects of adding increasing amounts of sodium acetate and sodium malonate to the culture medium of *Aspergillus niger* were investigated.

Experimental Procedure

Aspergillus niger (LBA 376) was maintained on potato dextrose agar slants. Spores were lightly scraped and washed from the slants with sterile medium. One ml of the spore suspension was used to inoculate 100 ml of sterile culture medium. One set of experiments used a medium containing from 0 to 5 g glucose and 0.2 g KH_2PO_4 ; 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.2 g NH_4NO_3 ; 5 g $\text{NaC}_2\text{H}_3\text{O}_2$; and 100 ml H_2O . In another, the medium contained 5 g glucose, 0.2 g NH_4NO_3 ; 0.2 g KH_2PO_4 ; 0.05 g MgSO_4 ; 100 ml H_2O and from 0 to 15 g of acetate or malonate. Control cultures contained no acetate or malonate. Cultures were incubated for 120 hours on a reciprocating shaker (180 revolutions per minute). Stir cultures were aerated and stirred for 120 hours. Cultures used for time studies were stirred and aerated in 600 ml of medium in a 1l flask.

At the end of the incubation period or at selected time points the mycelium was harvested by filtration through glass wool, resuspended in 15 ml absolute methanol, and sonified for 3 min (J-17A Sonifier, Branson Sonic Co.). The sonicate was diluted with an equal volume of 15% methanoic KOH, refluxed for 2 hours, and acidified with concentrated HCl. The fatty acids were extracted with hexane, washed with distilled water, and filtered through anhydrous sodium sulfate. After removing the solvent on a rotary evaporator, the fatty acids were methylated with diazomethane (14). The methyl esters were isolated on silica gel G thin layer chromatography plates, and separated, identified, and quantified by gas liquid chromatography using a 10-foot glass column packed with 5% DEGA on 60/80 mesh chrom GA/W; column temperature, 190°C; gas flow, 70 ml/min (Varian Aerograph 90-P).

Results and Discussion

Although Woodbine *et al.* (21) reported that the addition of increasing amounts of carbohydrates to the medium stimulated total fatty acid synthesis in *Aspergillus niger*, our results indicated that increasing the amount of glucose in the presence of 5% acetate did not affect fatty acid distribution in 120-hour cultures (Fig. 1).

The normal decrease in pH observed in cultures grown without acetate did not occur in cultures grown in a medium containing 5% glucose and 2.5% or 5% sodium acetate. Growth of the mycelium was markedly inhibited at these concentrations and completely inhibited at 15%. Romano and Kornberg (14) reported that the uptake of glucose in cultures grown in a medium with 100 mM acetate decreases as much as 80%. They suggested that acetyl-coenzyme A was responsible for the inhibition of hexose uptake. Apparently the acetate also inhibits the secretion of citric and gluconic acids which is characteristic of normal cultures.

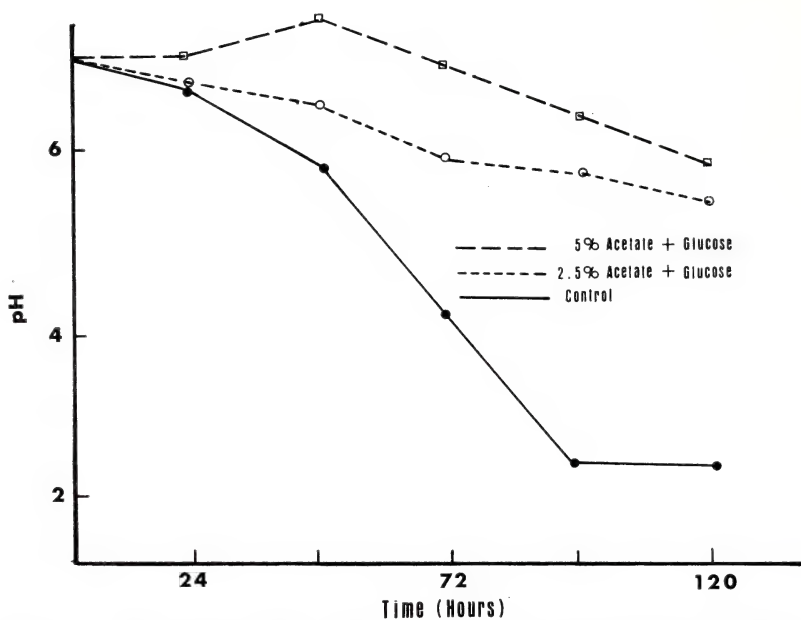


FIGURE 1. Changes in pH during growth of *Aspergillus niger* on different substrates.

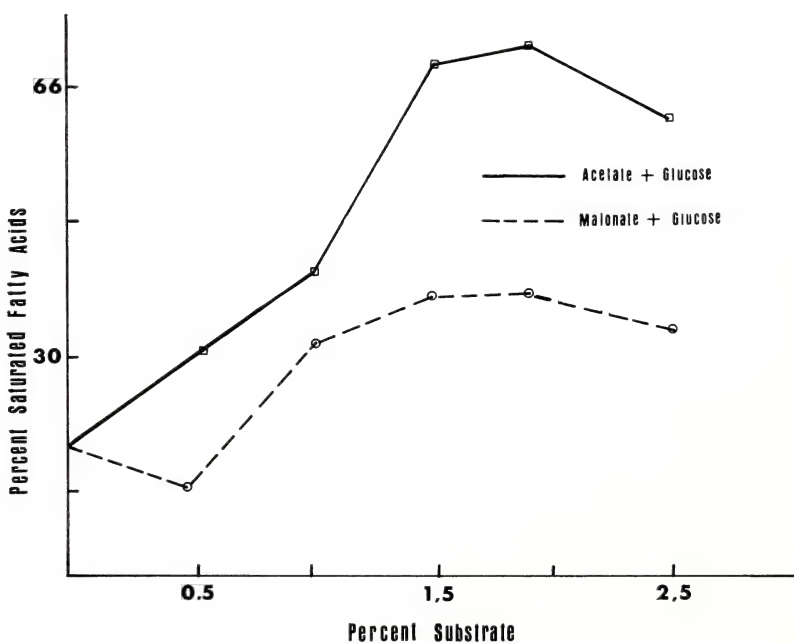


FIGURE 2. Per cent saturated fatty acids recovered from *Aspergillus niger* grown in the presence of acetate and malonate.

TABLE 1. *The effects of growing cultures two different ways¹.*

Culture	Saturated	Unsaturated
Control, Shake	20	80
2.5% Acetate, Shake	64	36
2.5% Acetate, Stir and Aerate	25	75

¹Harvested after 120 hours growth at 28°C.

The per cent saturated fatty acids produced by shake cultures grown in a medium containing 2.5% sodium acetate was 48% higher than that produced by control cultures (Fig. 2). A similar, but smaller (16%) increase was effected by the addition of sodium malonate. This effect could be reversed by stirring and aerating high acetate cultures (Table 1). Upon aeration the percentage of unsaturated fatty acids in the acetate culture was similar to the control.

However, when acetate was included in the medium, linolenic acid represented the largest component while linoleic acid was highest in the control (Table 2). Whether this difference in the distribution of unsaturated fatty acids was due to the increased availability of the acetate or the decreased availability of the glucose is not clear.

TABLE 2. *Per cent distribution of the major Fatty acids recovered from Aspergillus niger in a submerged medium containing varying concentrations of acetate¹.*

Per cent Acetate	Per cent Glucose	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
0.0	5	2	1	10	t	1	24	54	t
2.5	5	2	t	18	4	5	11	28	28
5.0	5	10	1	11	2	1	4	28	40
7.5	5	16	t	19	9	2	6	28	35
10.0	5	5	1	13	7	2	6	28	34
12.5	5	15	5	9	25	4	6	15	20
15-35	5	No Growth							

¹Grown at 28°C for five days in aerated stir cultures.

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PHYSICS

Chairman: **MALCOLM E. HULTS**, Physics Department,
Ball State University, Muncie, Indiana 47306

TORSTEN ALVAGER, Department of Physics,
Indiana State University, Terre Haute, Indiana 47809
was elected Chairman for 1972

ABSTRACTS

Effects of Pressure on Electronic Properties of Bismuth. **UWE J. HANSEN** Department of Physics, Indiana State University, Terre Haute, Indiana 47809, and **ELVIN M. COMPY**, US Naval Research Lab, Washington, D.C. 20390.—Experiments which study changes of electronic properties with variation in interatomic spacing are critical in their support of energy band calculations. The research reported here involves the first observation of cyclotron resonance and Alfvén wave propagation in Bismuth under pressures to 3 kilobars (Rev. Sci. Instr. 42:1215). The experiments are carried out at a temperature of 4° Kelvin using a standard 35 GHz bridge circuit. Effective mass decreases of the order of 6 per cent per kilobar are observed.

Photoelectric Photometry of an Eclipsing Binary Star System. **RONALD KAITCHUCK** and **NEWTON G. SPRAGUE**, Department of Physics, Ball State University, Muncie, Indiana 47306.—Many of the stars comprising our galaxy are members of a system of two stars in orbit about each other. The vast majority of these systems are far enough from the earth so as to appear only as a single point of light even in the largest of telescopes. However, if the orbital plane is seen edge-wise by an observer on the earth, during some portion of the orbital period an eclipse of one component will cause a drop in the observed light output.

The eclipsing binary system V566 Ophiuchi was studied in detail during the summer of 1971. Data were collected with a photoelectric photometer and the 12-inch Cassegrain telescope of the Ball State University Observatory. A complete light curve was obtained in two different spectral regions. The observations indicate that some event during the last 5 years has caused a change in the orbital period. There is also some indication that the color of the system is not as stable as was previously observed. It is hoped that the light curve will yield information as to the orbital elements. Standardization of the photometer system should yield the surface temperatures of the two stars.

Neutron Activation Cross Sections Using 2.8 MeV Neutrons. **DWIGHT E. MICHAEL** and **LEON M. REYNOLDS**, Department of Physics, Ball State University, Muncie, Indiana 47306.—A low-voltage type neutron generator-accelerator producing 2.8 MeV neutrons has been used to study neutron activation cross sections in a number of elements. There is a scarcity of good data in the literature because of the generally small values of these cross sections and special methods are required to increase the number of reactions occurring.

A relative measure of the neutron flux was made using indium standards. The efficiency of the detection system was experimentally measured by the use of standardized radioisotopes.

The product isotopes, produced by (n,γ) or $(n,n'\gamma)$ reactions, and their measured activation cross section were: ^{116m}In - $104 \pm 5\text{mb}$; ^{137m}Ba - $459 \pm 45\text{mb}$; ^{139}Ba - $5.48 \pm 0.47\text{mb}$; ^{128}I - $38.5 \pm 3.0\text{mb}$; ^{56}Mn - $4.28 \pm 0.34\text{mb}$; and ^{87m}Sr - $219 \pm 26\text{mb}$. Errors from 4.8 per cent for ^{116m}In to 12 per cent for ^{87m}Sr were determined.

The Numerical Lathe Fabrication and Theoretical Evaluation of an Electron Lens. JOHN SWEZ and JAMES WESTGARD, Department of Physics, Indiana State University, Terre Haute, Indiana 47809, and LARRY PLEW, Quality Evaluation Section, Crane Naval Ammunition Depot, Crane, Indiana.—The construction of an electron lens similar to that reported by A. V. CREWE (Quart. Rev. Biophys. 3:145) was attempted utilizing a numerical lathe. A computer program (APTURN) developed by the Cincinnati Research Corporation and used to generate control tapes for numerical lathes was modified to accept the equation used to generate the nonspherical surface of the lens. Although the optical properties of such a lens have been evaluated by A. N. Butler (Proc. 6th Int. Congr. for E. M., 1966), a partial evaluation of the optical properties was reported here. Computer calculated parameters were displayed on an oscilloscope interfaced to a PDP 8/I Computer.

Tayex: A Taylor Expansion Equation Solver. JAMES B. WESTGARD and DAVID G. PITTS, Department of Physics, Indiana State University, Terre Haute, Indiana 47809.—A digital computer program was written to implement a method of solving nonlinear simultaneous differential equations. The method was based on the Taylor expansion with an algorithm for finding the coefficients. It is extremely easy to use so that it should be useful in giving students confidence in being able to handle differential equations as they appear in course work. It will be especially useful for Lagrangian mechanics.

D-17B Minuteman Missile Computer Interfacing. C. T. WUNKER and P. R. ERRINGTON, Department of Physics, Ball State University, Muncie, Indiana 47306.—Ball State University has recently acquired via government excess property a type D-17B Minuteman missile guidance control computer. Hundreds of these mini-computers are being made available to colleges and universities throughout the United States. These computers, as received from the government, do not have usable input and output interfacing. To be useful as a teaching or research computer, interfacing must be designed and constructed. The computers and the interfacing now in use at Ball State University were described.

Growth of Single Crystals of Alkali Niobates. D. L. DEMOSS and C. C. SARTAIN, Department of Physics, Indiana State University, Terre Haute, Indiana 47809.—Single Crystals of lithium niobate and potassium niobate were grown from compounds made by solid state diffusion of the alkali carbonate with Nb_2O_5 . The compounds were melted and single crystals were pulled from the melt. Many experimental problems such

as phase separation of the melt and cracking of the crystal on cooling through its Curie temperature arose in our study. Our solution to these problems was discussed.

A Critical Study of the Various Factors Limiting Resolution in Solid State Detectors. K. E. WRIGHT and C. C. SARTAIN, Department of Physics, Indiana State University, Terre Haute, Indiana 47809.—A measurement of the intrinsic Fano factor for a particular Si (Li) detector was necessary to compare with theory. The Fano factor is defined as $\langle (N - \bar{N})^2 \rangle = F \bar{N}$, where N , ($\bar{N}$) is the (average) number of produced ionized pairs; $\bar{N} = E/\epsilon$, where E is the initial energy of the ionizing particle. The value of the intrinsic Fano factor was determined from the relation $\gamma^2 = \gamma_{\text{ion}}^2 + \gamma_{\text{col}}^2 + \gamma_{\text{noise}}^2$ and by linear extrapolation of γ^2 for the infinite field strength from the dependence of γ^2 on the reciprocal detector voltage. This measured value of the intrinsic Fano factor will ultimately determine whether or not carbon x-rays (277 ev) can be detected. The Si (Li) detector will provide chemical analysis composition when coupled to an scanning electron microscope.

Calculus Without Differentials. PHILLIP S. MARCUS, Department of Mathematics, Indiana University at South Bend, South Bend, Indiana.—The differential notation of Leibniz has recently been superceded in popularity among mathematicians by the dot-prime notation of Newton and LaGrange, and the abstract operator D notation of Cauchy. Leibniz differentials are still used in integral calculus. Karl Menger showed that differentials can be replaced by the unbelievably simple device of giving an explicit symbol— j —to the identity function. Examples were given of definite and indefinite integrals and seperable differentiable equations which were usually integrated by manipulation of differentials, but the usual manipulation of differentials was replaced by Menger's improved notation for substitution of functions.

NOTE

Neutron Activation Analysis of a Metal Button from the Site of Fort Harrison. TORSTEN K. E. ALVAGER and RALPH A. LLEWELLYN, Department of Physics, and ROBERT E. PACE, Department of Anthropology, Indiana State University, Terre Haute, Indiana 47809.—Among the artifacts recovered by the archeologists from the apparent site of the Fort Harrison Cemetery were 7 metal buttons. Severe corrosion had destroyed those features that might otherwise reveal unit, rank, and date of manufacture. With the view of searching old metal specification records, the metallic composition of the best preserved of the buttons was examined by the non-destructive technique of neutron activation analysis. Preliminary analysis using the ISU 2-curie Pu-Be neutron source indicated that more complete examination would be of interest. The detailed analysis was performed by irradiating the button for 48 hours in the General Atomics 10-curie Pu-Be neutron source in San Diego, California. Following irradiation, the gamma-ray activity produced was analyzed using a 36 cc Ge (Li) detector and a 4096 channel analyzer.

On the basis of the observed gamma-ray energies, the elements positively identified as being present in the button are: Cu, Zn, Au, and As. It is estimated that copper makes up 89 per cent of the button, zinc and arsenic a few per cent each, and gold less than 1 per cent. What appear to be gold flecks are visible on the surface of the button, indicating that the gold may have been used as a plating or gilding. The button seems to be constructed in layers with zinc overlying a copper base. This observation together with the relatively higher copper content indicates the button was probably not made of alloyed copper and zinc (brass). Tests are currently underway using an electron microprobe to "map" the distribution of metals in the button in order to obtain additional composition details.

The presence of arsenic is not particularly surprising, but the relatively large amount, perhaps as much as 5-7 per cent, is. Arsenic occurs with copper in some ores, and it may be that the high arsenic percentage could serve to identify either the source of the ore or the refining process. While no investigation along these lines has as yet been started, a preliminary search of old metal specification records has been conducted. Unfortunately, no satisfactorily close match has been found; thus, at this writing we are not able to positively identify the button analyzed as being associated with Fort Harrison. However, the examination is continuing and may lead to additional results later.

OTHER PAPER READ

The Decay of ^{71}As . A. E. RAINIS and J. R. VAN HISE, Department of Physics, Tri-State College, Angola, Indiana.

A Study of the Rate of Absorption of Methylated Mercury by *Carassius auratus*

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Abstract

Radioactive tracer techniques were used in determination of the rate of absorption of a methyl mercury compound by goldfish. Goldfish were retained for specified times in test concentrations of CH_3HgCl containing commercially-prepared γ -active Hg^{203} . It was found that the fish concentrated the mercury by a factor of 2×10^2 in the 36-48 hours required for saturation.

Introduction

The problem of mercury poisoning has recently been one of intense interest in the United States and other countries (1, 2). The degree of contamination, the biological effects, the allowable safe amounts in our waters, the methods of contamination, and many other questions pertaining to mercury and its compounds remain, at best, only partially answered. This paper is primarily concerned with the rate at which a particular mercury compound is absorbed by goldfish at various concentrations.

Monomethyl mercury was used because of its solubility in water and because it is the generally accepted compound produced by anaerobic bacteria in river and lake bottoms (4).

Measurement

Known concentrations of the CH_3HgCl with γ -active Hg^{203} was used as a radioisotope tracer. This isotope was chosen because the γ -emitted could be easily detected without destroying the fish. To keep the fish alive during the time required to measure the γ -activity (a few minutes), the fish were placed in a container of fresh running water.

The detector arrangement was calibrated and adjusted using a pulse height analyzer. The initial adjustments were made with gamma rays from a Cs^{137} source to obtain a suitable energy calibration. The intensity calibration was accomplished using a known quantity of Cs^{137} and Na^{22} and a scaler counter. Several different levels of contamination were investigated. These were 5 ppb, 50 ppb and 500 ppb. Six fish were placed in each container.

Results

The curves shown in Figure 1 is of the form $C = C_M(1 - e^{-\lambda t})$, where C is the concentration, C_M is the maximum concentration achieved and λ is a factor related to the rate of absorption. For the 5 ppb sample

$C_M = 0.73$ ppm and $\lambda = 0.96 \text{ hr}^{-1}$ and for the 50 ppb sample $C_M = 6.9$ ppm and $\lambda = 0.108 \text{ hr}^{-1}$. With these fits, one half of the final concentration is reached in only 7.2 hours and 6.4 hours for the 5 ppb and 50 ppb concentrations, respectively. In addition to the absorption rate found, it was discovered that on two different occasions a concentration of 500 ppb produced 100% fatality in that particular group of fish within 24 hours.

A study of the rate of de-absorption was also attempted but was not completed. The reason we did not complete this study was that our Hg^{203} with a 47-day half-life decayed so rapidly that meaningful numbers could not be obtained. A reasonable assumption for the biological half-life in fish is 500 days during normal activity (3). The only conclusion we could draw was that the rate of de-absorption was much greater than the rate of decay of Hg^{203} .

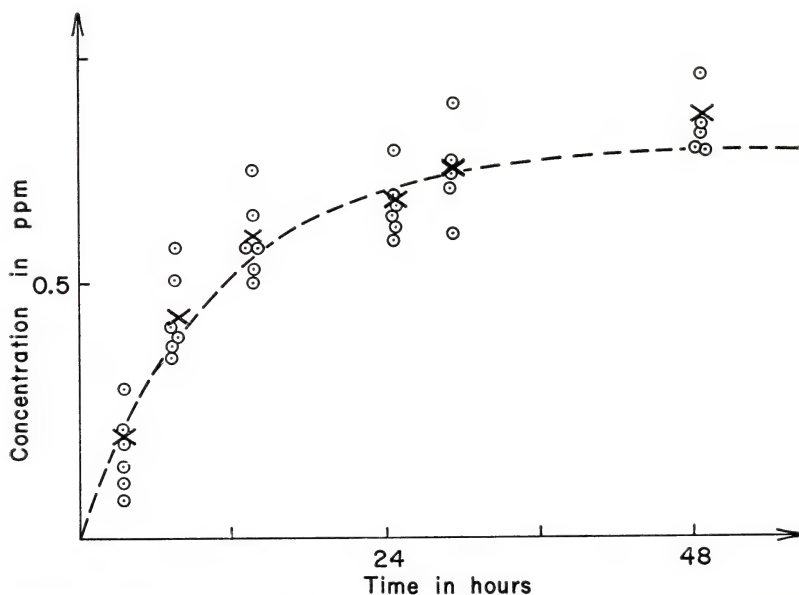


FIGURE 1. Result for aquatic concentration of 5 ppb. ($\odot$ represents the concentration in the individual fish; $\times$ represents the fish weight averaged concentration.)

Acknowledgements

The authors wish to thank Mike Berry and Carl Hanger for their assistance during this experiment.

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PLANT TAXONOMY

Chairman: FAY KENOYER DAILY, Botany Department, Butler University, Indianapolis, Indiana 46208

GERALD GASTONY, Department of Botany, Indiana University, Bloomington 47401, was elected Chairman for 1972

ABSTRACTS

The Computerization of the Edward Lee Greene Herbarium (NDG) at Notre Dame. THEODORE J. CROVELLO, Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556.—Edward Lee Greene (1843-1915) is a fascinating and important figure in American botany. Throughout his career he was an active field botanist and named over 4,400 genera, species and subspecies new to science. The University of Notre Dame possesses his herbarium of 70,000 specimens. Label and other data are being captured on punched paper tape and input into a computer. The procedures, biological value and types of output being produced were discussed. Because Greene left no collection notebooks, it is only from a computerized rearrangement of his specimen label data that his itinerary can be obtained. Also, he often did not indicate which specimens were type specimens. The specimen data and the data on what species he named can be used to pinpoint some type specimens and to generate a list of missing type specimens. The computer is seen as a valuable tool in assisting the taxonomist in his information retrieval problems and as a means to enhance the value of this Indiana resource.

The Origin of Seed Planting. CHARLES B. HEISER, JR., Department of Botany, Indiana University, Bloomington 47401.—Recent archaeological investigations have revealed a great deal about the origin of agriculture, but the question as to why man ever changed his mode of subsistence remains unanswered. A religious motive has been suggested for the origin of animal domestication, and in a little known paper published in 1897 Grant Allen postulated that the sowing of seeds for crops began as an adjunct to the primitive burial system. While Allen's proposal has some merit, I am more inclined to look for the origin of seed planting as a consequence of the "first fruits" or "last sheaf" ceremonies of primitive seed collectors. Primitive man believed that spirits existed in plants and as a propitiation to the spirit the first fruits were returned to the fields. This magico-religious act would make the seeds safe for mortals to eat and at the same time assure a beautiful harvest in future seasons. Such a hypothesis would also offer a possible explanation for the rapid improvement of the early cultivated plants, in that man would return some of the best seeds to the field instead of eating them. Thus one might postulate that artificial selection began to operate with the offering of seeds to the spirits of the plants.

Wild Flowers of Franklin County and Indiana. LLOYD and ADELE BEESLEY, Cedar Grove, Indiana.—In the *Flora of Indiana* by Charles C. Deam,

two species of *Chelone*, Turtlehead, are listed with variants. We have found and photographed the following:

1) *Chelone oblique* var. *speciosa* Pennell and Wherry. Rose Turtlehead. Grows in wet woods and less frequently in springy places in woodlands.

2) *Chelone glabra* var. *typica* Pennell. White Turtlehead. Five varieties of this species are cited for Indiana which grow in wet woods, springy places about lakes, along streams and in marshes. However, we do not have all of them at this time.

A Biosystematic Study of *Polygonum ramosissimum* and *Polygonum tenue*¹

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Abstract

Plants collected in Wisconsin during September 1970 were identified as *Polygonum ramosissimum* and *P. tenue* according to the taxonomic character established by Styles for European species of genus *Polygonum* and adapted for North American species of this genus by Mertens and Raven. The somatic chromosome numbers for these two species were determined to be 60 for *P. ramosissimum* and 30 or 32 for *P. tenue*. These counts were compared with chromosome numbers of species of section *Polygonum*, all of which have a somatic number of either 40 or 60. Chromatographic analysis of free amino acids and secondary substances further suggests that *P. ramosissimum* has amino acids similar to those of other species in section *Polygonum*. Chromatographic data for *P. tenue* is somewhat less conclusive in assigning this species to a section of genus *Polygonum*.

Genus *Polygonum*, section *Polygonum* (*Avicularia*) consists of a number of morphologically similar plant species. Individuals within each species vary morphologically according to local edaphic and climatological conditions. This has led to a general confusion among taxonomists who have been attempting to identify field and herbarium specimens, on the one hand, and to systematists, on the other, who have been attempting to assign natural rank to the various species of *Polygonum*. This study reports on an attempt to establish the natural relationships of two *Polygonum* species to section *Polygonum* by using cytological and chemical parameters.

Research in the 1960's brought taxonomic clarification to some species within section *Polygonum*. Styles (9) defined the four species of the *Polygonum aviculare* aggregate found in Britain (*P. aviculare*, *P. arenastrum*, *P. rurivagum*, and *P. boreale*), and three other species found elsewhere in Europe (*P. maritimum*, *P. raii*, and *P. oxyspermum*) in terms of fruit and perianth characteristics and chromosome number. He found achene texture, color, shape and size, and inflorescence position to be stable and conservative traits. Mertens and Raven (5), using the biosystematic approach of Styles (9), studied the *P. aviculare* complex in North America and were able to devise a workable key for eight species of section *Polygonum*. Savage and Mertens (7) studied six species of *Polygonum* found in Indiana and Wisconsin by using the parameters established by Styles. Moore, Mertens, and Highwood (6) summarized cytotaxonomic data on various species within this same section. These workers compared chromosome number with the taxonomic characters decided upon by Styles and later researchers, to determine if indeed a plant with a certain combination of fruit and perianth characteristics consistently displayed the same chromosome count.

¹This study was supported by a grant to Dr. Mertens from the Society of the Sigma Xi.

The present investigation is concerned with examining *Polygonum ramosissimum* and *P. tenue* to determine if they are to be included in section *Polygonum*. The two species, collected in Wisconsin, were keyed according to the following taxonomic couplet established by Savage and Mertens (7):

Achene surface predominantly smooth and shiny but may have stippled or striated edges; inflorescences appear to be terminal, the flowers being more-or-less clustered at the ends of stems among reduced leaves or bracts; perianth tightly oppressed to the achene, which has three equal concave sides.

Achene surface completely smooth, achenes dark reddish brown, sharp angled; calyx yellowish green, plant large [3-10 dm], much branched *P. ramosissimum*

Each face of black, trigonous achene smooth and shiny but bordered with striated or stippled margin; plant slender [1-4 dm], stiff or wiry; leaves linear *P. tenue*

Materials and Methods

All plant material was collected in Wisconsin at two sites along the Wisconsin River on September 27, 1970. Both species were collected in a sandy beach habitat. *Polygonum ramosissimum* was found 2¼ miles due west of Lone Rock in Richland Co., Wisconsin. *Polygonum tenue* was gathered in Upham Woods in Juneau Co., Wisconsin.

Viable achenes were removed from the plants and germinated on moist filter paper in petri dishes. Chromosome counts were made of root tip and apical meristem cells treated with the mitotic poison, oxyquinoline, and stained with aceto-orcein.

Chromatographic patterns of the two plants under study were compared with patterns made by other species of *Polygonum*, section *Polygonum*. Single dimensional paper chromatograms testing for the qualitative distribution of free amino acids and secondary substances among the compared plants were run to see if a related pattern developed. It was assumed that related species would have similar, but not necessarily identical, chromatographic patterns, and that different plants of the same species would consistently display the same patterns.

A mixture of 0.3 g of plant root, stem, and leaves was pulverized in a mortar and then transferred to a vial with 2 ml of 80% methanol. After a drop of 1 N hydrochloric acid was added, the vial was corked and allowed to stand at room temperature for 24-48 hours before the extract was used to streak the chromatography paper.

Single dimensional chromatograms were developed by the descending method using 46 X 57 cm Whatman No. 1 chromatographic paper. The plant extracts were applied to the paper with a 10- μ l pipet. Generally 100 μ l of plant extract were needed to get readable results. The chromatograms were developed for 24 hours, using a solvent mixture, described by Smith (8), consisting of 12 parts n-butyl alcohol, 3 parts glacial acetic acid, and 5 parts distilled water.

The chromatograms in which the free amino acids were to be detected were sprayed with a ninhydrin aerosol bomb. The chromatograms were dried in the dark for 24 hours before observations were made. Secondary substances were detected by placing unsprayed chromatograms under long-wave ultraviolet light in the presence of ammonia vapors.

Data

Definite chromosome counts were obtained for *Polygonum ramosissimum* from these plants:

P. ramosissimum Michx., Richland Co., Wisconsin, Sept. 27, 1970, G. M. Brooks No. 89 (BSU). Somatic count of 60.

P. ramosissimum Michx., Richland Co., Wisconsin, Sept. 27, 1970, G.M. Brooks No. 75 (BSU). Somatic count of 60.

Photographs of the chromosomes found in cells of these two plants were taken (Fig. 1). Diagrammatic line drawings of the cells counted were made because all chromosomes do not appear equally well at the focal level from which the photomicrographs were taken (Fig. 2).

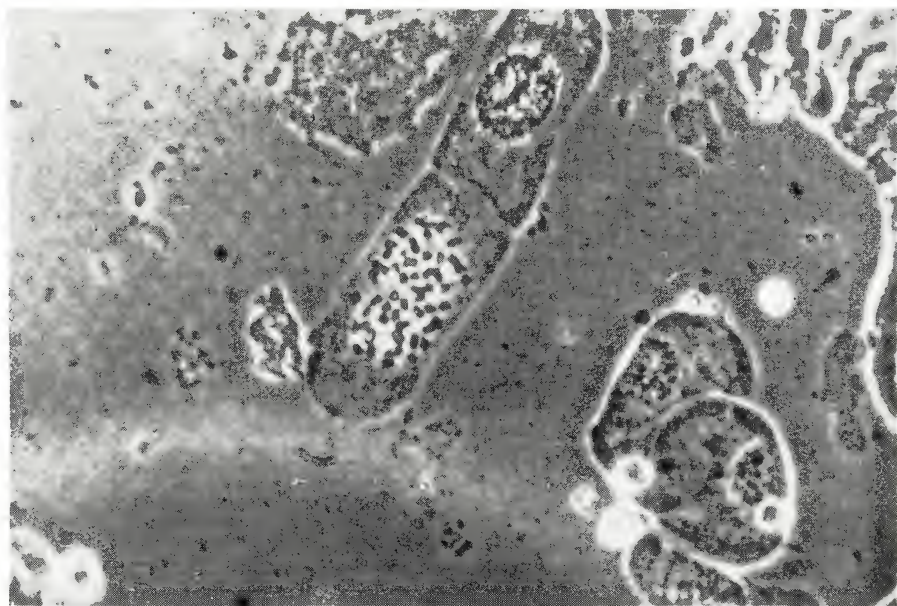


FIGURE 1. Photograph of chromosomes from *Polygonum ramosissimum*, G. M. Brooks No. 89. Chromosome number $2n=60$ (1000 X Magnification).

Tentative counts were obtained from the following specimens of *Polygonum tenue*:

P. tenue Michx., Juneau Co., Wisconsin, Sept. 27, 1970, G.M. Brooks No. 209 (BSU). Somatic count of ca. 30.

P. tenue Michx., Juneau Co., Wisconsin, Sept. 27, 1970, G.M. Brooks No. 216 (BSU). Somatic count of 30 or 32.

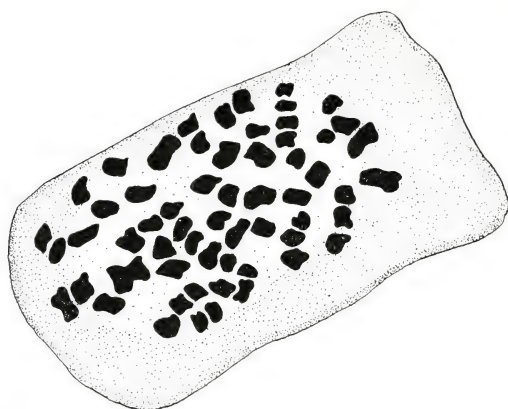


FIGURE 2. Diagrammatic line drawing of cell of *Polygonum ramosissimum* shown in Figure 1. Chromosome number $2n=60$.

The chromatographic procedure described previously was repeated with at least five different specimens of each species studied. The specimens of each species were found to display consistent chromatographic patterns. The following specimens were used as representative samples in preparing the chromatograms from which the data in Tables 1 and 2 were determined:

P. aviculare L., Great Bras d'Or, Nova Scotia, August 17, 1969,
D. Jones No. NSJ 15 (BSU).

P. erectum L., Porter Co., Indiana, August 29, 1966, A.D.
Savage No. 58-30 (BSU).

P. ramosissimum Michx., Richland Co., Wisconsin, Sept. 27, 1970,
G. M. Brooks No. 89 (BSU).

P. tenue Michx., Fulton Co., Indiana, August 20, 1966, A.D.
Savage No. 50-1 (BSU).

TABLE 1. R_f values of the qualitative distribution of free amino acids for four species of *Polygonum*.

Color	<i>P. aviculare</i>	<i>P. erectum</i>	<i>P. ramosissimum</i>	<i>P. tenue</i>
Pink	.06	—	.06	—
Purple	.12	.11	.12	.10
Purple	.14	.14	.14	.15
Purple	.19	.18	.18	—
Purple	.26	.24	.26	.26
Purple	.31	.31	.32	.34
Yellow	.36	.35	.36	.37
Purple	.43	.40	.40	.40
Purple	—	.47	.46	—
Purple	.52	.52	.53	.53
Purple	.58	.59	.60	—
Purple	.65	—	.66	—

A single dimensional paper chromatogram was developed for *P. aviculare*, *P. erectum*, *P. ramosissimum*, and *P. tenue*. It was sprayed with ninhydrin to develop the free amino acids which had migrated down the paper from the sites of origin. A second single dimensional chromatogram was developed with the same four plants and was placed under long-wave ultra-violet light in the presence of ammonia vapors to highlight the secondary substances. Table 1 gives the R_f values of the qualitative distribution of the free amino acids for the four plants; Table 2 shows the same for the secondary substances.

TABLE 2. R_f values of the qualitative distribution of secondary substances for four species of *Polygonum*.

<i>P. aviculare</i> R_f Color	<i>P. erectum</i> R_f Color	<i>P. ramosissimum</i> R_f Color	<i>P. tenue</i> R_f Color
.37 yellow	.47 blue	.53 green	.45 yellow
.46 blue	.58 yellow	.60 yellow	.55 yellow
.55 yellow	.69 yellow	.70 yellow	.62 blue
.68 yellow	.82 blue	.85 blue	.69 yellow
.77 yellow			.80 blue

Discussion

Species of genus *Polygonum* section *Polygonum* generally give somatic chromosome counts of 40 or 60. Styles (9) determined the somatic number 40 for *P. arenastrum* and 60 for *P. aviculare*, while Mertens and Raven (5) determined the somatic number of 60 for *P. marinense*. Löve and Löve (4) reported that *P. erectum* and *P. fowleri* each has a somatic number of 40, and these counts have been confirmed by Moore, Mertens, and Highwood (6). It may be that the basic chromosome number in this group of plants is $x=10$, and that those species with 40 chromosomes are of tetraploid origin, while those with 60 are hexaploids. This suggests that *P. ramosissimum* might also have a somatic chromosome count of 40 or 60 if it is to be included in section *Polygonum*. The determination that *P. ramosissimum* indeed does have a somatic count of 60 agrees with this cytotaxonomic requirement. Löve and Löve (4) published a somatic chromosome number of 20 for this species collected in Manitoba. However, voucher specimens were not kept for cross-checking purposes, thus calling their finding to question.

Polygonum tenue collected for this study had a somatic chromosome count of 30 or 32. This departure from a count of 40 or 60 (which numbers seem to be generally characteristic of plants in genus *Polygonum*, section *Polygonum*) may suggest that *P. tenue* belongs to another section of the genus. This would be especially true if the count proves to be 32 rather than 30. Löve and Löve (4) reported this plant to have a somatic count of 20. Again, no voucher specimens were kept to verify their findings for *P. tenue* collected in Minnesota. Mertens and Raven (5) commented that *P. tenue* was morphologically similar, from the standpoint of its fruit and

perianth characteristics, to other species of section *Polygonum*, but that this plant had pollen of the type found in section *Duravia*. (2). Thus, palynological data tend to support the contention that *P. tenue* should be assigned to a section of the genus other than section *Polygonum*.

Alston and Irwin (1) were able to separate closely related species of the genus *Cassia* (Caesalpinia Family) by using paper chromatographic techniques. Jones and Mertens (3), by using the same technique, were able to qualitatively distinguish among a number of species of *Polygonum* section *Polygonum*. The data in the present investigation (Tables 1 and 2) show that *P. ramosissimum* has basically the same qualitative distribution of amino acids as do the two established species of section *Polygonum*, *P. aviculare* and *P. erectum*.

Although the chromatogram of amino acids for *P. tenue* indicated that this species has many spots in common with the other three species studied, certain repeatable differences in the chromatograms were noted:

- 1) To obtain a readable chromatogram for *P. tenue* specimens, the extract had to be four times as concentrated at the site of origin as for any of the other species investigated.

- 2) The color intensity of the various chromatogram spots for *P. tenue* was always less than for comparable spots of the other species.

- 3) Although the size of the streak of origin of the *P. tenue* chromatogram was comparable to that used for the other species, the size of the spots in the chromatogram were consistently smaller than comparable spots for the other species.

It may be argued that the chromatogram of *P. tenue* differs from those of the other three species only in lacking certain spots and that this could be due to less diversity in its genome in response to a more restricted habitat. However, the three points cited above would seem to argue for the uniqueness of the chromatogram of *P. tenue*.

Secondary substances are so termed since they are not involved in basic energy transfer or catabolic activity of plant tissues. These compounds (alkaloids, terpenoids, and polyphenols) are stable by-products and so can be used as chemical taxonomic parameters.

Although certain spots in the chromatograms of secondary substances of the different species seem to be identical (e.g., .68 yellow in *P. aviculare* and .69 yellow in *P. erectum*), the overall qualitative distribution of secondary substances was unique for each species. This suggests that secondary substance analysis may be used to establish speciation, since each of the four species studied had a different overall chromatographic pattern and various plants within a species displayed a consistent pattern.

Conclusions

- 1) *Polygonum ramosissimum*, with a somatic chromosome count of 60, seems to be similar to established species of section *Polygonum* all of which have either 40 or 60 as their somatic chromosome number.

2) Since the tentative somatic chromosome counts of 30 or 32 for *P. tenue* were made from collections at one site, and since they do not agree with counts reported in the literature, fresh specimens of *P. tenue* containing viable achenes should be collected and a definite chromosome number determined.

3) Chromatographic studies indicate that the overall qualitative free amino acid distribution for *P. ramosissimum* is similar to the distributions shown by species of *Polygonum* in section *Polygonum*.

4) *Polygonum tenue* has quite a different overall qualitative amino acid distribution due to: a) the greater concentration of extract required in order to get readable chromatograms, b) the much weaker color intensities for the various spots, and c) the smallness of the spots in comparison to comparably colored spots of the three other species at the same R_f locus on the chromatogram.

5) The overall qualitative distribution of secondary substances is unique for each species with various specimens of the same species giving the same pattern.

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Studies in Indiana Bryophytes XIV

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Abstract

Two additional species and two additional varieties have been found to occur in Indiana since the publication of Studies in Indiana Bryophytes XIII (15): *Atrichum undulatum* (Hedw.) P. Beauv. var. *oerstedianum* (C. Müll.) Crum; *Grimmia pulvinata* (Hedw.) Smith; *Isopterygium distichaceum* (Mitt.) Jaeg. & Sauerb.; and *Pohlia annotina* (Hedw.) Lindb. var. *loeskei* Crum, Steere and Andrews. Specimens of each have been deposited in the Herbarium of DePauw University. One species, *Grimmia indianensis* (Sayre) Crum (3), originally *G. trichophylla* Grev. var. *indianensis* Sayre (12), has now become a synonym of *G. pulvinata* (Hedw.) Smith. The total number of known species of mosses in Indiana is presently 228, varieties 39, and forms 9, a total of 276 kinds.

The American Bryological and Lichenological Society 1970 foray was held in West Central Indiana, including habitats in Putnam and Parke Counties. One species, *Isopterygium distichaceum* (Mitt.) Jaeg. & Sauerb., and one variety, *Pohlia annotina* (Hedw.) Lindb. var. *loeskei* Crum, Steere and Anderson, were collected by two foray members, which are first records for Indiana. Another collection is that of a species (9) or a variety (4) which has been present in eastern United States indefinitely, within the species *Atrichum undulatum* (Hedw.) P. Beauv. *A. oerstedianum* (C. Müll.) Mitt. was not known to occur in the United States until the research on *Atrichum* published by Ireland (9).

On July 22, 1971, Keith Hoss, an Army Corps Engineer, and I visited Rocky Ford, on Big Pine Creek, in Warren Co., Indiana, searching for *Grimmia indianensis* (Sayre) Crum, in the area in which I collected the type, Nov. 27, 1937. The search was in vain. All of our collections were *G. alpicola* Hedw. The next day Mr. Hoss returned to Rocky Ford, crossed Big Pine Creek, on to Mud Pine Creek, a tributary to Big Pine, and climbed Table Rock in the creek bed. Table Rock has a flat top and steep sides. Mr. Hoss estimated this block of sandstone, a remnant of erosion (11) to be ca. 8 feet high, 50 feet long, and 30 feet wide, and exposed 6-7 feet above the water in the stream. On the flat top surface, there is a pothole, 2 feet deep and 10 feet in diameter. On this rock Hoss made several collections of *G. pulvinata* (Hedw.) Smith,¹ a

¹From a letter from H. A. Crum, Oct. 22, 1971: "I am convinced that *Grimmia indianensis* is a synonym of *G. pulvinata*. I would perhaps never have thought of it, except for the several collections you recently sent from Warren County and the type locality. It appears that both typical *G. pulvinata* and typical *G. indianensis* grow in the same locality, the latter probably no more than a shaded expression with less obviously contorted leaves and a poor development of hairpoints on the leaves. (I have seen a similar development from Scotland.) The specimen which you sent could be called *G. indianensis*, but it merges in its variations almost completely into typical *G. pulvinata*."

first record for Indiana, and *G. laevigata* (Brid.) Brid. We are indebted to Howard Crum, Univ. of Michigan, for making or checking determinations.

Bryaceae

Pohlia annotina (Hedw.) Lindb. var. *loeskei* Crum, Steere, and Anderson. [Var. *decipiens* Loeske, in Grout (8).] (Fig. 1). Plants in loose tufts, pale green, yellowish green, or green, not glossy when dry,

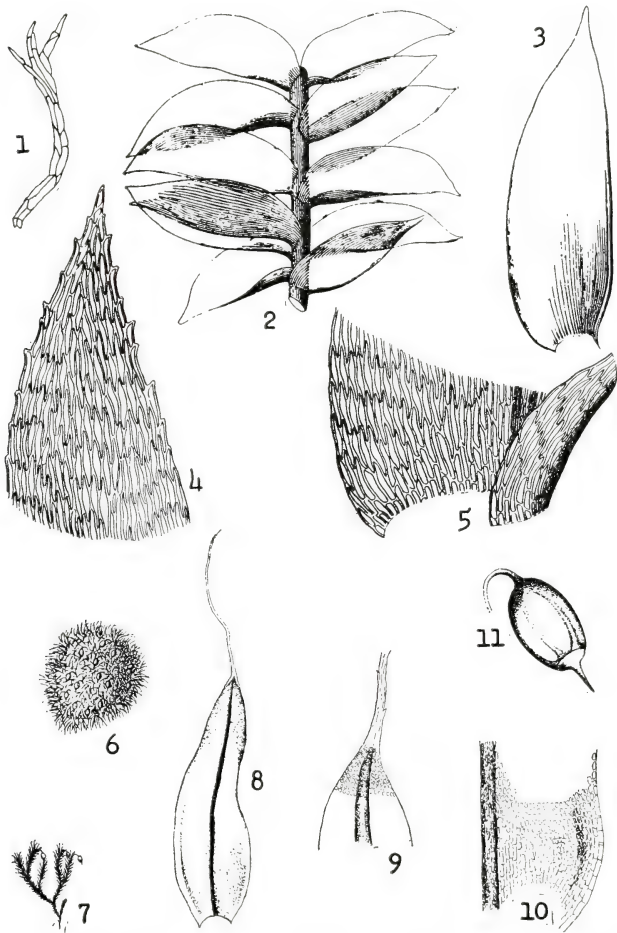


FIGURE 1. *Pohlia annotina loeskei* (Conrad, 2, Fig. 107e), gemma. FIGURES: 2-5. *Isopterygium distichaceum* (Grout, 6, Pl. 43C, from Sullivan, 13, Pl. 67). 2. Portion of foliated axis. 3. Leaf. 4. Upper portion of leaf. 5. Base of leaf. FIGURES 6-11. *Grimmia pulvinata* (Grout, 7, Pl. 13C, from Braithwaite, 1, Pl. 48C.) 6. Habit. 7. Plant, with sporophytes. 8. Leaf. 9. Leaf apex, with base of hair point. 10. Basal portion of leaf. 11. Upper seta, with capsule. *Atrichum undulatum oerstedianum* is not illustrated because Welch (14), Fig. 21, is sufficient. [Figures by permission, from the late H. S. Conrad and the late A. J. Grout.]

to 2 cm high, with some radicles. Stems slender, erect, simple or branched from the base, flexuose reddish at base. Leaves erect-spreading, frequently decurrent, ovate-lanceolate, lanceolate below, narrowly lanceolate above, the upper ones erect or nearly so. Apices mostly acute, occasionally acuminate. Margins of leaves plane to slightly recurved, especially near base in dry leaves, entire below, toothed above. Costa strong, commonly percurrent, yellowish to reddish at base. Cells of leaves narrowly rhomboidal to linear, to $80 \times 7\mu$, the walls moderately thick. Gemmae in leaf axils, especially the upper ones, commonly 2-5 each, hyaline or yellowish to green, elongated, twisted-vermiform, generally with 3-4 acute erect leaf-points. Dioecious. Usually sterile. Seta red, flexuose, slender, to 2 cm long. Capsule reddish brown, inclined, horizontal, or pendulous. Urn long-pyriform or broadly oval, to + 3 mm long, with a tapering neck, $\pm$ the length of the urn. Operculum conic, apiculate. Annulus removable. Peristome teeth yellowish, slender. Segments hyaline, slender, carinate, split along the keel. Cilia in pairs. Spores $\pm 18\mu$ in diameter.

Habitat: Moist, sandy soil, especially among sandstone rocks.

Indiana distribution: Parke Co.: wet faces of Mississippian sandstone gorge wall, in Sword Moss Gorge, ca. 14 miles W of Greencastle, Norton G. Miller 5929 (DPU, NCU).

Grimmiaceae

Grimmia pulvinata (Hedw.) Sm. (Fig. 6-11). Plants in small, round tufts, appearing grayish green due to long hyaline hair points. Upper leaves oblong-lanceolate, to 2 mm in length, abruptly narrowed into a long, hyaline, flexuose, serrate hair point. Both margins revolute from base nearly to tip, or plane and bistratose at apex. In upper-lanceolate leaves, hair points nearly as long as blades. Upper cells of leaves rounded, incrassate, the lower ones thin-walled, quadrate, except 5-6 rows near costa rectangular. Autoecious. Calyptra mitrate. Capsule exserted, pendent, $\pm$ ovoid. Operculum rostrate. Seta arcuate, 2-3 times the length of capsule.

Habitat: Common on dry rocks, also on walls and roofs, usually at lower altitudes.

Indiana distribution: Warren County.

Hypnaceae

Welch (14) considers the genus *Plagiothecium* B.S.G. in the family of Hypnaceae. Crum *et al.* (5) regard *Plagiothecium* as a genus in the family of Plagiotheciaceae. Ireland (10) in his revision of the genus *Plagiothecium* treated it as a genus in the Hypnaceae.

According to Ireland (10), the Indiana species in Welch (14) should be revised as follows. *Plagiothecium denticulatum* (Hedw.) B.S.G. and *P. roesanum* B.S.G. are correct; *P. micans* (Sw.) Paris = *Isop-*

terrygium tenerum (Sw.) Mitt.; *Plagiothecium muellerianum* Schimp. = *Isopterygium muellerianum* (Schimp.) Jaeg. & Sauerb.; *Plagiothecium deplanatum* (Sull.) Spruce = *Taxiphyllum deplanatum* (Sull.) Fleisch.; *Plagiothecium geophilum* (Aust.) Fleisch. = *Taxiphyllum taxirameum* (Mitt.) Fleisch.; *Plagiothecium sylvaticum* (Brid.) B.S.G. is a European species and has been excluded from North America. "Most of the North American plants named *P. sylvaticum* are depauperate *P. roeseanum* B.S.G."

Isopterygium distichaceum (Mitt.) Jaeg. [*Plagiothecium subfalcatum* Aust. in Grout (6)] (Fig. 2-5). Plants in thin, soft, intertangled mats, bright green to yellowish green, glossy. Stems usually prostrate, sometimes ascending to erect, appearing flattened because of complanate leaves. The leaves often distant, appearing distichous, spreading, obliquely attached to axis, subfalcate, many apices pointing toward base of axis or substratum, frequently asymmetric, sometimes subcylindrical, at times undulate, oblong-lanceolate to ovate-lanceolate, not decurrent, $0.3-1.8 \times 0.2-0.6$ mm. Apices acute or sometimes broadly short acuminate. Margins plane or narrowly recurved below, entire to serrulate below, the apices serrate-dentate. Leaf cells usually smooth, sometimes papillose on lower surface by projecting cell angles in apices. Median cells linear, $48-100 \times 4-7\mu$. Propagula commonly in leaf axils of the apical portions of the axes, elongated, twisted-vermiform, 2-4 rows of cells wide, to 0.5 mm long, with 1-5 acute apical points. Calyptrae, antheridia, archegonia, and sporophytes not known in North America.

Habitat: In shade, on sandstone cliffs in crevices of rocks, and on banks of soil and humus.

Indiana distribution; Putnam Co., Hoosier Highlands, on Mansfield sandstone, ca. 14 mis. S of Greencastle, Nancy G. Slack 851 (DPU). Verified by R. Ireland.

Polytrichaceae

Atrichum undulatum (Hedw.) P. Beauv. var. *oerstedianum* (C. Müll.) Crum. *Atrichum undulatum* in Indiana was described and illustrated by Welch (14), based on collections from 50 counties. Ireland (9) states that most of the eastern North American specimens of *Atrichum* in the herbarium of the Museum of Natural Science, National Museums of Canada named *A. undulatum* are *A. oerstedianum* (C. Müll.) Mitt. and that *A. undulatum* is not as common as once believed.

The following is a brief summary of Ireland's description. Plants 2-6 cm high. Dry leaves usually undulate, twisted and crispate, lingulate to lanceolate, $5-9 \times 0.8-1.6$ mm, acute, ending in a tooth. Margins bordered $\pm$ throughout with 2-3 rows of narrow, linear, thick-walled cells, the border usually bistratose; upper margins toothed, singly or doubly. Costa subpercurrent to percurrent, toothed on lower surface from midleaf to apex, lamellae on upper surface. Lamellae 4-6, often undulate, 2-4 cells high, occasionally 6. Leaf cells smooth or minutely papillose. Median cells rounded or irregularly

angled, $12-31\mu$ in longest dimension, averaging less than 17μ in shortest dimension. Calyptra hispid at tip. Dioecious. Seta 1.5-4 cm long, 1-3 per perichaetium. Capsule cylindric, usually inclined and subarcuate. Urn 4-7 mm long. Operculum obliquely rostrate, 2-3.5 mm long. Spores $12-17\mu$ in longest dimension.

Ireland states that *A. oerstedianum* is our native species and one of the common species in eastern North America and that *A. undulatum* has been introduced, probably from Europe, and rarely collected on this continent. Ireland adds that the latter prefers a dry, weedy habitat, such as weedy roadside ditches and the former occurs in wet habitats, especially on humus and soil along stream banks and inundated areas.

Crum (4) reduces this species to the synonymy of *Atrichum undulatum* and makes the var. *oerstedianum* (C. Mull.) Crum. He states, "Without sex organs or capsules, the var. *undulatum* and the var. *oerstedianum* are impossible to distinguish (except sometimes by size and habitat)." The var. *undulatum*, at least in some plants, is monoecious, and the var. *oerstedianum* is dioecious.

Habitat: On soil or humus, mostly in shaded situations; often in wet areas along streams or occasionally at margins of swamps.

Indiana distribution: Putnam Co., Fern Cliff, on bank, $\pm$ 9 mis. W of Greencastle, Nancy G. Slack 763, 778 (DPU). Determined by R. Ireland.

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The Effects of Preservation Time on Chromatographic Patterns in *Equisetum hiemale*

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Abstract

A study was made to determine if the age of a herbarium specimen of *Equisetum hiemale* L. had any effect on the chromatographic patterns. Specimens of *E. hiemale* were analyzed employing descending paper chromatography. The amino acid and phenolic patterns were of significant value in determining if the chromatographic patterns varied as the herbarium specimen aged. The fluorescent phenolic compounds of *E. hiemale* were analyzed by placing the chromatograms under long wave ultraviolet light. The free amino acids were detected by spraying the chromatograms with ninhydrin.

The chromatographic patterns showed that the age of the oldest herbarium specimen investigated had no effect on its patterns.

The purpose of this study was to determine if variations occur in the free-amino acid and phenolic chromatographic patterns of *Equisetum hiemale* L. samples taken from herbarium specimens as compared with those obtained from fresh material.

Since the technique of paper chromatography appeared, it has contributed significantly to systematic studies (3). The works of Alston and Irwin, Heywood, Smith and Levin, and others have firmly established the study of "secondary constituents" as a valid taxonomic method (1, 4, 7).

Analyses can be carried out on fresh plants from the field, or greenhouse transplants. In the absence of fresh material, the investigator may have to resort to using herbarium specimens. Since some taxa are exceedingly rare, and some are difficult to grow under greenhouse conditions, it is significant to know whether detectable differences exist between freshly-dried specimens and ones which have been dried for long periods of time. Some workers have stated that no significant differences exist (7). Others suggest that analyses are best carried out on fresh materials (6).

Methods and Materials

Herbarium specimens of *Equisetum hiemale* were obtained from the Missouri Botanical Garden Herbarium and the Field Museum of Natural History. Those materials had been preserved for periods up to 144 years. Fresh material was collected in Richmond, Indiana.

Comparisons of free-amino acid and phenolic composition were made by means of 1-dimensional paper chromatography. Extracts were prepared by powdering dried internodes and soaking the materials in methanol:water:hydrochloric acid (7.9 : 2 : 0.1) for 24 hours at room temperature. One hundred microliters of each sample were applied to 23 × 57 cm sheets of Whatman #1 paper using the spot method. The chromatograms were run using n-butanol:acetic acid:water (12 : 3 : 5) as the solvent. Following air drying at room

temperature, the chromatograms were viewed under long wave ultra-violet light in the presence of ammonia vapor. Those spots which fluoresced but did not show a ninhydrin reaction were taken to represent phenolics (1, 2, 5). Subsequently the chromatograms were sprayed with ninhydrin for the detection of free-amino acids and related substances (1, 2, 4, 5, 7).

Results

The chromatographic results of horsetails which had been preserved for periods of time ranging from four to 144 years were compared chromatographically with freshly collected materials (Table 1).

TABLE 1. *Equisetum hiemale* used in chromatography and their sources.

Date Collected	Locality	Collector	Reference Number
Sept. 1970- Feb. 1971	Richmond, Indiana	L. C. Brown	P1, A1
Jun. 1966	Cooke City, Montana	Robert G. Stolze	P2
Nov. 1958	Lafayette, Louisiana	W. D. Reese	P3
Jul. 1955	Iantha, Missouri	E. J. Palmer	A4
June 1946	Cumberland, Rhode Island	E. J. Palmer	P5, A5
Oct. 1935	Greenwich Pt. Pennsylvania	John M. Fogg, Jr.	P6
Jul. 1926	Spearfish, South Dakota	Herman E. Hayward	P7
Aug. 1911	Hull, Canada	John Macoun	P8, A8
Jun. 1902	Amesbury, Mass.	Walter Deane	P9
Oct. 1890	Flor de Maria, State of Mexico	C. G. Pringle	P10
May 1880	Preble, New York	J. H. Schueffer	P11, A11
1871	Koenigberg, Germany		P12
1862	Hock Creek, Georgia	Dr. Arthur Schott	P13
May 1858	Easton, Pa.	T. Green	P14
April 1847	Mexico	G. Gregg	P15
1826	Frankfort, Germany	G. Engelmann	P16

The ultraviolet patterns viewed in the presence of ammonia vapor were consistently characterized by the presence of nine compounds. These compounds are designated by number in Figure 1. Compound 2 was present in all except P13. Compound 1 was detected in all samples except P11, P12, and P16. Compounds 5 and 7 existed in all samples except P9 (Fig. 1).

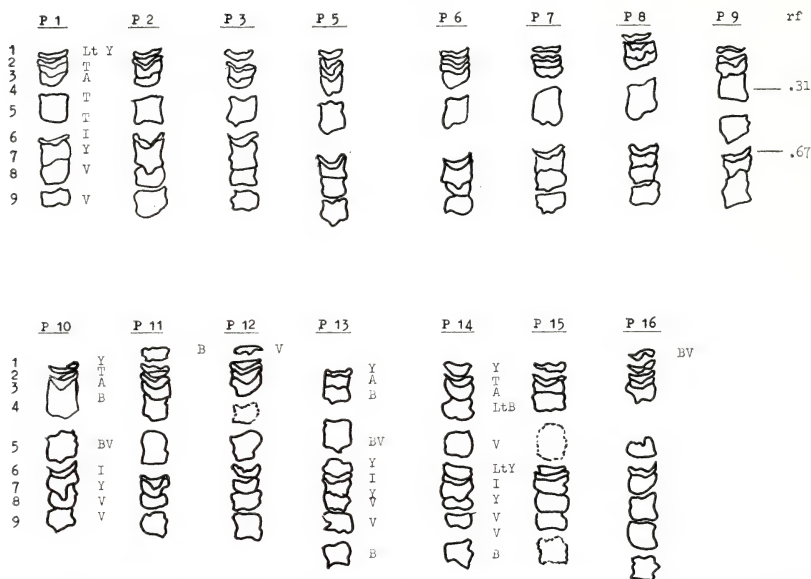


FIGURE 1. Patterns of *Equisetum hiemale* in ultraviolet light (A=avocado, B=blue, BV=blue violet, I=ivory, LtB=light blue, LtY=light yellow, T=turquoise, V=violet, Y=yellow).

Chromatograms sprayed with ninhydrin showed remarkable consistency in free-amino acid composition. All samples revealed nine compounds except A4 in which spot # 1 was not observed (Fig. 2).

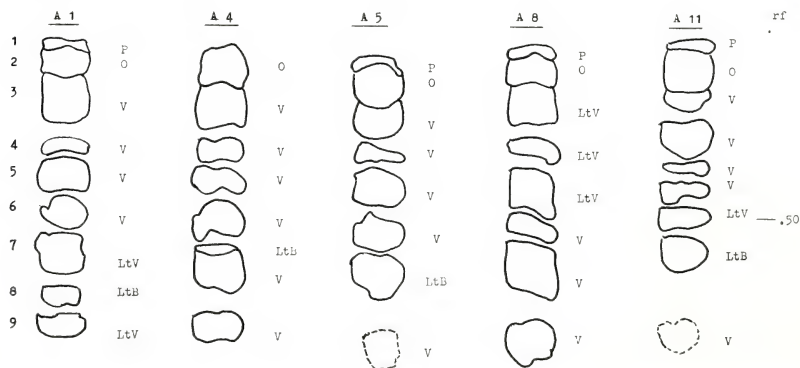


FIGURE 2. Amino acid patterns of *Equisetum hiemale* (LtB=light blue, LtV=light violet, O=orange, P=pink).

Discussion and Summary

The results of this study indicate that the place and aging of herbarium specimens have little effect on the free-amino acid and phenolic composition in *Equisetum hiemale*.

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Comments on a Check List of Indiana Algae: Purpose and Problems

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Abstract

A check list of Indiana algae by county has been compiled from the literature appearing from 1929 to 1971. This is the fifth in a series of publications initiated by C. M. Palmer who covered the algal reports of 1875 to 1928. A few additions to this period have been made. The purpose of the check list and some problems in its use and preparation are discussed.

In 1929, C. M. Palmer (1) provided a check list of Indiana algae based upon his own research and publications appearing from 1875 to 1928. Additions were included in three later papers (2, 3, 4). Distribution maps for each taxon were filed at Butler University. When Dr. Palmer moved to Ohio in 1950, the work was continued by the present author and William A. Daily. The first check list since then covering the 1929 to 1971 period has been compiled and will be distributed by the author upon request.

In the interest of continuity, algae are listed essentially in the same style as that used by Palmer except for literature citations. The genus, species and infraspecific names are followed by the county where the algae were found and with the literature citation in parenthesis (Table 1).

TABLE 1. *Indiana algae listed by county and bibliographic reference number.*

GROUP 1. BLUE-GREEN ALGAE

AGMENELLUM Breb. *Agmenellum thermale* (Kütz.) Dr. & Da.:

Fulton (43), Grant (43), Kosciusko (43), LaGrange (43), Marion (43), Morgan (43), Parke (43), Putnam (50). *A. quadruplicatum* Bréb.:

The purpose of this work is to inventory the algae occurring in the state and to give distribution by county. This information is useful as an historical document and may help relocate specimens in herbaria or in nature. The latter is possible because some algae may persist in the same or nearby habitats for many years. The interest in geographical occurrence anticipates possible reflected ecological patterns. A. C. Smith (5) summed up the value of systematics as follows: "Agriculture, medicine and all phases of human economy ultimately depend upon natural living organisms of which no real understanding can be had without a basic inventory and knowledge of spatial and environmental relationships."

Nomenclature in the check list is the same as in the literature cited. This was necessary because specimens are not available as a basis for all reports. This precludes restudy to produce a uniform,

modern classification. There is also a lack of agreement on the classification of many algae. Herbarium specimens are not available, in part, because of the following differing concepts or methods in taxonomy.

Specimens preserved by drying and storing in an herbarium are sometimes criticized as worthless, so none were kept by the authors. Others prepared liquid-preserved specimens which have dried and have been discarded. However, some taxonomists have studied the effects on algal morphology of varying conditions of the habitat, differing methods of collecting, handling and restoring specimens, and can recognize most algae from well-preserved dried specimens demonstrating the necessary stages for identification. This ability is necessary for a monographer employing the type method or in applied microscopy.

Various techniques have been applied to algal taxonomy during the 1929 to 1971 period. These include the study of cultured algae, the use of scanning and electron microscopes and various advances in genetics, physiology and biochemistry. Many of these studies have made substantial contributions.

However, some methods require cultured algae free of contaminating organisms, and only cultures are cited. It has been found that algal morphology is sometimes different after isolation in artificial media. It has been suggested that this may be due to genetic changes induced by shock of isolation or unnatural growing conditions, undetected contamination by a second alga which may become dominant later, the mixed genetic constitution of the original isolate or other undetermined factors. If the mechanisms of inheritance and genetics have not been compared in cultured algae and natural populations, genetic studies based on cultured algae alone may result in classification without general application due to the above difficulties.

On the other hand, algal morphology may be very uniform under the cultural conditions employed. Results from the study of a few of these specimens may not reveal the wide range of morphological variation that is apparent in a study of a large number of specimens from nature under varying habitat conditions.

It is, therefore, recommended that herbarium specimens of natural collections (or soil collections, if applicable) be prepared as a basis for all algal reports. Comparison of adult forms can be made with cultured material to detect morphological change. Resting forms can be recultured from soil for comparison. Classification based on culture characteristics should indicate the specimens seen by the code of the culture collection.

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SCIENCE EDUCATION

Chairman: KENNETH UHLHORN, Science Teaching Center
Indiana State University, Terre Haute, Indiana 47809

FREDERICK K. AULT, Ball State University, Muncie, Indiana
47306, was elected Chairman for 1972

Uses of a Student Responder System. LLOYD L. BODIE, JR., Planetarium Director, Vigo County School Corporation, Terre Haute, Indiana.—Traditionally, educational procedures have required that the instructor have a limited maximum number of students within the classroom. The Student Response System (SRS) has made it possible to bring some of the most important features of the small teaching classes to large group instruction. This paper described the use of SRS by a class of 65 junior and senior students from high schools in Vigo County School Corporation, enrolled in Astronomy, at the Allen Memorial Planetarium. All students had a science and mathematical background. Topics discussed included the strengths and weaknesses of this system as used in our program; the description of the Responder System—the instructor's console, the student units, and the central electronics unit printer; the student's operation of the responder system; the value to the student; and some typical questions asked.

An Audio-tutorial, Independent Study Program for the Elementary Teacher. CONRAD CARLYLE HINDS, Department of Education, Ball State University, Muncie, Indiana 47306.—The purpose of this study was to develop and test an audio-tutorial, independent study program on simple machines to meet the individual needs of elementary in-service teachers.

The research was designed to determine how well in-service teachers could learn the specific content involved using this particular approach. Advantage was taken of the teaching skills and methods which teachers employ in their own classes. Pre- and post-tests of the Smith Mechanic Test were administered.

Two samples were drawn from the population of elementary teachers in the Science 590 extension and summer classes at Ball State University. There was a control group of 29 elementary teachers and an experimental group of 48 elementary teachers. The experimental group used the audio-tutorial program. The control group received classroom instruction over the specific content involved.

The difference in means between groups was examined for significance using a two-tailed t-test. No significant difference was found on the pre-test. A significant difference beyond the 0.01 level was found on the post-test ($t = 2.87$, 75 d.f.). Each group was considered separately for significance in the mean gain. The control group showed significance at the 0.05 level ($t = 2.05$, 28 d.f.). The experimental group showed significance beyond the 0.001 level ($t = 13.54$, 47 d.f.).

It was concluded that the audio-tutorial program afforded a much better mode for practice over the specific content involved than did classroom instruction. It was recommended that the program be used in conjunction with elementary pupils to measure any increase in teacher competency more accurately; that the program be used in a teaching situation other than the extension class to measure retention; that the program be used as one mode of presentation in the Science 590 classes; and that administrators institute this program into schools as part of continuing in-service training.

Training Supervising Teachers to Evaluate and Counsel Pre-Service Teachers on the Basis of Teaching Techniques Derived From Methods Courses. STANLEY S. SHIMER, LINDA B. KNIGHT, and HAROLD H. JAUS, Science Teaching Center, Indiana University, Bloomington, 47401.—The Indiana University, Bloomington Campus, Triple "T" Program is a professional year internship for pre-service elementary teachers which combines student teaching with basic methods courses. This program assumes that the techniques taught in methods classes are implemented in the classroom. However, it was found that the terminology used in the methods class, indeed, even the teaching techniques covered in the course were unfamiliar to many of the classroom supervising teachers. Thus, there was very little reinforcement of the method topics used by student teachers in the classroom. A package of instructions was developed for the supervising teachers to acquaint them with the topics dealt with in the methods courses and to aid them in reaching competency in evaluating a student teacher's use of these techniques. This enabled supervising teachers to evaluate and counsel the student teachers. It also provided the student teachers with much needed reinforcement and feedback on the use of teaching techniques.

The package consisted of 9 hours of performance-based-instruction on techniques selected from the Science Methods Curriculum, *e.g.*, performance objectives, question asking skills, formative evaluation, teacher responses, systematic instruction and sequencing. Formative Post Tests were designed for each unit and summative evaluation was administered at the end of the 9 weeks. Data indicated that supervising teachers are more able to accurately evaluate and counsel pre-service teachers after receiving this package of instruction.

An Environmental Science Conservation Course. H. DOUGLAS SHOCK, Cowan High School, RR 4, Muncie, Indiana 47304.—An environmental science conservation course for the high school level was developed to give students a basic understanding of their environment, of the environmental problems which are facing each of them and to help each student discover his role in making the environment a place worth living. The writer taught the course for 2 years before formalizing it by writing a creative project for the degree of Master of Science at Ball State University. To accomplish this, the following study units were planned: Life Designs, Conservation in Perspective, Ecology, Populations, Soil Resources, Minerals and Energy, Water Resources, Air

Resources, Plant Resources, Animal Resources, Resource Relationships, Recreation, Community Planning, and Careers.

The course was designed to use a wide variety of learning experiences rather than from a single source, such as a textbook. Books, pamphlets, library materials, films, resource people, and field experiences relative to the course of study were planned to enhance the student's awareness of his environment. Group work was encouraged throughout the course to help build a willingness for cooperation.

NOTE

Reading Habits of Prospective Secondary Earth Science and Geography Teachers. WILLIAM D. BROOKS, Department of Geography and Geology, Indiana State University, Terre Haute, Indiana 47809.—At Indiana State University, within the Department of Geography and Geology and in cooperation with the Division of Teaching, all geography and earth science teaching majors are required to take a 2-credit hour course entitled "The Teaching of Geography in the Secondary Schools" (G393). A methods course, G393 meets 4 days each week for 8 consecutive weeks. This 8-weeks period represents the first half of the professional semester while the second half is spent in a student teaching experience under local school and university supervision.

Juniors and seniors prior to the student teaching experience and prior to attaining professional status should be in the process of organizing in-depth knowledge not only in a chosen content area but also in educational theory and practice. It seems obvious that an upper semester junior or senior enrolled in a program leading to professional status should have developed or be in the process of developing a sophisticated reading habit.

The purpose of this study was to examine the reading habits of prospective secondary teachers of geography or earth science.

To test empirically student reading habits, all students enrolled in G393 for six consecutive semesters were asked to submit a one page report each week on anything they had read that could in any way be related to the teaching of geography or earth science. No other directions were given nor were restrictions imposed. Each time the course was given a number of class periods were spent examining and discussing a variety of sources that could be used to acquire knowledge in earth science, geography and education.

First, a total of 258 reports were submitted which represented 113 different sources. Twenty-six of the 113 sources were considered professional-educational journals. Thirty-eight books were critiqued, thus 64 of the 113 sources can be considered excellent sources for acquisition of knowledge.

Second, of the 258 reports 188 were from important journals. Add 39 book reports (one book reported twice) for a total of 157 reports gathered from first-rate information.

Third, 88 reports were gathered from newspapers, layman journals (*Newsweek*, *Time*, etc.) and government agencies. The rest of the reports, 13 in number, were placed in that "catchall" category other.

OTHER PAPERS READ

"ISCS" or Implementing Science in a Confusing Situation. JACK BREWER, M.S.D. of Perry Township.

Our Model for I.S.C.S. Based on Five Years Experience. CHARLES E. RICHARDSON, M.S.D. of Lawrence Township.

SEE System for Ecological Education

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and

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Abstract

The System for Ecological Education (SEE) is a new systematic approach to life-centered environmental education which employs a scope and sequence format. It includes grade level teaching materials for K-6, and for grades 7-12 a scheme of eight topics of concern which can be integrated into existing curriculums.

SEE provides a unifying framework within which the environmental input of all educators may become cumulative. It is ecologically comprehensive and forms the basis for college level teacher training. It establishes for K-12 educators minimum ecological understandings for each year of study. It suggests a route to take to bring about these understandings; yet it allows full latitude for the teacher to exercise initiative and imagination.

Because SEE is a system, it requires teacher direction to assure a smooth flow, and this direction may be used to make sure that the child becomes a participant in the educational experience. Students are expected to learn by doing. The laboratory for learning is their own local surrounds.

Scope and Sequence—Grades 1-6

The scope and sequence charts, one for each grade, are based on environmental concepts developed by Roth (2); the U. S. Department of Agriculture, Forest Service (3); and the Indiana Department of Public Instruction (1). There has been substantial grouping of material for simplification, and most concepts are presented in language appropriate to the grade level in which they are used. The concepts selected for each grade are those which are comprehensible and which contribute to the progressive grade by grade development of the horizontal thought threads. The six threads are: 1) *Living Things*, 2) *Needs of Living Things*, 3) *Meeting Needs and Wants*, 4) *Problems from Meeting Needs and Wants*, 5) *Solving Problems*, and 6) *Man's Moral Responsibility*. It is important to understand that in these threads the material becomes increasingly more complex in each successive grade. Across the time span of the child's education, each thread remains intact; and at the completion of high school, building is still taking place on the understandings introduced in kindergarten or in one of the lower grades.

K-12 Grade and Subject Area Teaching Materials

Kindergarten

The system presents 25 pages of material which is divided into three subject areas: 1) *Using the Senses*; 2) *Introducing the Physical Environment—Air, Soil, Water, and Sun*; and 3) *Living Things*. Included in each area are experiments, outdoor activities, games, and a bibliography guiding the teacher to books, films, filmstrips, pictures, charts, and other visual aids.

Grades 1-6

Individual grade level charts have been developed. These expand the concepts found on the scope and sequence charts for each grade. The expansion sometimes involves more than one sub-concept. It employs the same horizontal threads used in the scope and sequence chart. Grade level charts are made up of one vertical column for the concept or sub-concept and six vertical columns with the following headings: 1) *Introducing the Concept*; 2) *Developing the Concept*; 3) *Films, Filmstrips, & Slides*; 4) *Other Visuals*; 5) *Publications*; and 6) *Other Resources & Suggestions*.

In many columns of their grade charts, teachers will be guided by an asterisk (*) to refer to a separate guide book. In it they will find suggested activities to help develop the concept or written material to further their own understanding of the concept.

Grades 7-12

The concepts for these grades are presented in four vertical columns 1) *Title*; 2) *The Natural Laws (Governing Factors)*; 3) *Man Apart from Nature (Problems Created)*; and 4) *Man a Part of Nature (Solving Problems)*.

Each of eight topics is treated in every vertical column. The study of the first topic, *How Many People are Enough?*, for example, involves consideration of three things in the column entitled *The Natural Laws*: 1) Population change is the product of biotic potential and environmental resistance. 2) Living things increase in number to the level the environment will permit. Man's ability to modify the environment does not make him an exception. 3) The Earth's carrying capacity is finite for one and all species.

In the column *Man Apart from Nature*, students consider the need for restrictive laws and regulations which arises as the population increases. They also find proof that as numbers of people increase, the need for food, fiber, and minerals (energy in particular) expands.

In the column *Man a Part of Nature*, they give careful study to man's obligation to function rationally in a system with his culture and the biophysical environment.

There are seven other subjects covered by this same horizontal approach: 1) *Consuming the Fruits of Technology*; 2) *For a Beautiful World*; 3) *Preserving Man as a Sensitive Creature*; 4)

Man Needs Wilderness; 5) Man and Adaptation; 6) Conserving our Natural Resources; and 7) Man and the Environment.

Examples of Horizontal Developmental Threads

The K-12 study of living things is an example of the systematic development of a child's understanding of increasingly complex material by building each year on the base erected the previous year. This study is initiated in kindergarten as the children use their five senses to learn about life around them. In succeeding years, they discover how living things differ and how they are alike. They explore the world of living things and learn that there are also similarities and differences in the ways living things acquire the things they need and want. Continuing to build their understanding, they use similarities and differences in form and function to divide plants and animals into groupings. Before completion of grade six, they learn to construct and use various kinds of taxonomic keys. The depth of understanding they achieve either as individuals or as a class depends on their interest and ability. The study of living things continues thru the upper five grades. Each of the eight topics which are integrated into the existing curriculums in these grades is concerned with one or more aspects of living things. The K-6 foundation studies of living things are prerequisite to a full understanding of the eight topics.

Another example which demonstrates both the systematic development of a concept thread and student involvement also starts with the study of living things in kindergarten. However, this thread branches in grade one from the route taken in the previous example to become resource rather than biologically oriented. The children develop in class their ideas of the difference between needs and wants. In subsequent grades, they find out how the natural resources of planet Earth are used to satisfy our needs and wants. They then seek local evidence of problems which can be traced to the fact that each year we are using resources to meet extensive and growing demands of more and more people. The students appraise the influence of advertising, particularly the creation of desire for more wants, and the resultant increase in resource consumption. A field trip to the local solid waste disposal facility focuses on the serious problem of disposal of the child's own throwaways. This "needs and wants" thread continues into the eight topic areas and broadens the base from which they are studied.

Throughout the whole system, man's moral responsibility to Earth and all its living things is stressed. The child is guided to explore ways his own behavior influences the quality of his environment. He forms his own ideas of what behavioral patterns are required of individuals and both small and large groups if there is to be a quality life for all living things.

The System for Ecological Education is designed to be an educational tool with which to shape attitudes and values and to construct patterns of behavior which will make each citizen an environmental asset.

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SOIL SCIENCE

Chairman: RUSSELL K. STIVERS, Agronomy Department,
Purdue University, Lafayette, Indiana 47907

CHRISTIAN JOHANNSEN, Agronomy Department, Purdue
University, Lafayette, Indiana 47907, was elected Chairman
for 1972

Abstracts

Soil Colloids and Behavior of Pesticides in Soils.¹ JOE L. WHITE and MARIBEL CRUZ,² Purdue University, Lafayette, Indiana 47907.—The interaction of pesticides with soil is largely dependent upon the nature and properties of the surfaces of the colloidal components of the soil. Interactions of organic molecules with surfaces of aluminosilicate minerals have been studied by infrared and other spectroscopic techniques. These studies have provided evidence for the mechanisms involved in adsorption, bonding, and degradation of several groups of pesticides by inorganic soil components. The role of the higher degree of dissociation of water molecules on clay surfaces ("surface acidity") in the protonation and hydrolysis of chlorotriazines has been established by this work. These studies also suggest that competition between pesticide molecules and water molecules for adsorption sites, together with changes in the environment of the pesticide molecules resulting from physical adsorption processes, may explain desorption and volatilization losses.

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The Precision Associated with Sampling Frequencies of Total Particulate at Indianapolis, Indiana. DALE E. PHINNEY and JAMES E. NEWMAN, Agronomy Department, Purdue University, Lafayette, Indiana 47907.—The frequency distribution of total suspended particulate matter for Indianapolis, Indiana, was examined to determine the precision associated with a given sampling scheme. By assuming a basic log_e-normal distribution, a theoretical set of confidence intervals about the geometric mean was derived for random sampling. Verification of the log_e-normal distribution was made for particulate matter in Indianapolis. Application of the derived confidence intervals revealed that for a 30-day period 20 samples must be taken to ensure that the 90 per cent confidence interval will be within 10 per cent of the geometric mean. Analysis of the records for 19 sampling locations showed that only 2 sites possessed sufficient data to allow monthly climatological evaluation over the period 1968-1970.

OTHER PAPER READ

The Good in Pollution. A. J. OHLROGGE, Purdue University.

High Rates of Urea Fertilizer for Corn (*Zea mays* L.) on Two Soils, 1969-1971¹

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Abstract

Five rates of nitrogen from urea with accompanying rates of agricultural limestone were compared for continuous corn on Fincastle silt loam soil and on Chalmers silty clay loam soil. The effects of the urea were similar in both soils, but they were more pronounced on the Fincastle soil which was lower in both organic matter and in cation exchange capacity. In 1969 on the Fincastle soil, the highest rate of urea furnishing 1344 kilograms per hectare of nitrogen, when applied the day before planting, reduced stands of corn to 32 per cent and yields of corn grain to 37 per cent of those which received 336 kilograms per hectare of nitrogen. In 1970, there was no stand, vegetative growth or meaningful grain yield reductions when the same high rates of fertilizer were plowed under on the same plots in November prior to seeding in May. In 1971, soluble salts in the Fincastle soil were more than doubled, height of plants was reduced 20 per cent, and soil pH was reduced 0.9 unit with the 1344 kilograms per hectare rate of nitrogen as compared with the 336 kilograms per hectare rate of nitrogen.

Introduction

Powell and Webb (3) found that in the third year of continuous corn, yields began to decrease above 336 kg per ha (300 lbs per A) or 448 kg per ha (400 lbs per A) of nitrogen. They attributed this reduction to accumulation of salts and to decreases in soil pH. The purpose of this research, which is similar to that of Powell and Webb, is to compare high rates of N for continuous corn.

Methods

Two soil areas approximately 1 km (0.6 mile) apart were selected on the Purdue University Agronomy Farm. The Chalmers silty loam, classed as a Typic Arguaquoll, had been well fertilized and limed, as well as tile drained, and had been cropped to corn and soybeans (*Glycine max* L.) for several years. The plow layer had a cation exchange capacity of 32.5 meq per 100 g of soil, contained 4.4% organic matter, had 185 parts pp 2m of Bray No. 1 phosphorus (P) and had 675 pp 2m of potassium (K) as determined by the Purdue Soil Testing Laboratory. Soluble salts were low with a specific conductance of 19×10^{-5} mhos. This area is depressional. The other soil, a Fincastle silt loam, is classed as a Aeric Ochraqualf. It had received moderate rates of fertilization, had been well limed, and had been cropped to continuous corn for several years. Cation exchange capacity of the plow layer was 16.3 meq per 100 g of soil, and the organic matter content was 2.3%. It had 100 pp 2m of Bray No. 1 phosphorus (P) and 285 pp 2m of available potassium (K). Soluble salts were low with a K value of 16×10^{-5} mhos. Tile drainage was inferior to that in the

¹Journal Paper No. 4593. Purdue University Agricultural Experiment Station.

Chalmers soil. Land leveling was used on the Fincastle soil prior to the initiation of this experiment to make the 2-4% slopes more nearly uniform.

The plot layout was a randomized complete block design with four replications of five treatments on each soil. Treatments were no N and four rates of N from fertilizer grade urea (45%) and rates of limestone as shown in Table 1. Soil samples from all plots were taken in May before applying treatments and in August of 1969, 1970 and 1971. Lime requirement was determined by use of the Shoemaker, McLean and Pratt buffer (4). Soil pH was determined using a 1:1 soil to water ratio and a Corning glass electrode pH meter. The agricultural limestone from the Delphi Limestone Company in 1969 had an average of 36% of the particles passing through a 60-mesh sieve and 95% through an 8-mesh sieve. The 1970 limestone from the Newton County Stone Company had 38% passing through a 60-mesh sieve and 95% through an 8-mesh sieve. The urea was broadcast one day before planting for the 1969 crop and in the previous

TABLE 1. *Relation of rates of urea fertilizer and limestone application to soil pH, soluble salts, and nitrate nitrogen on two soils, 1969-71.*

Kg/ha of N Applied Annually	Soil pH			M.Tons/ha Limestone Added		Soluble Salts Specific Conductance K (Mhos x 10 ⁻⁵)			Nitrates ppm
	5-28-69	8-13-69	8-3-71	'69	'70	8-13-69	6-29-71	8-3-71	6-20-71
Fincastle silt loam									
0	6.1	6.1	6.4	4.5	4.5	18	15	20	1
168	6.2	6.1	6.4	4.5	4.5	20	21		1
336	6.2	5.9	6.2	9.0	6.1	26	36		12
672	6.2	5.6	5.7	6.7	7.2	55	62		35
1,344	6.1	5.4	5.3	9.0	8.1	115	99	78	88
LSD 05 for rates	Not Sig.	.2	0.3				12	yes ¹	13
Average	6.1	5.8	6.0			47	47	49	
Chalmers silty clay loam									
0	6.4	6.5	6.5	0	0	15		16	
168	6.6	6.5	6.6	0	0	22			
336	6.7	6.5	6.6	0	0	45			
672	6.5	6.4	6.3	4.5	2.7	68			
1,334	6.4	5.9	5.9	6.7	5.2	115		36	
LSD 05 for rates	0.3	Not Sig.	0.5					yes	
Average	6.5	6.4	6.4			53		26	
LSD 05 for soils	Yes ²	yes ²	Not Sig.					yes ²	

¹Yes means averages for the different rates of N are significantly different from each other at the stated or greater probability.

²Yes means averages for the two soils are significantly different from each other at the stated or greater probability.

November for the 1970 and 1971 crops. In 1969 all fertilizer was broadcast after plowing disked, or field cultivated for incorporation. For the 1970 and 1971 crops broadcast limestone and fertilizers were plowed under in the previous November, and field cultivation and spike tooth harrowing were used for spring preparation for planting. In all years the urea was applied last just prior to disking or plowing. In 1969, 1,190 kg per ha (1,065 lbs per A) of 0-11-21 (percentages of total N, available P, and available K) were applied to all plots. For the 1970 and 1971 crops 975 kg per ha (870 pounds per acre) of 0-11-21 fertilizer were applied each year to all plots.

Broadcast aldrin was incorporated into the soil in 1969 to control soil insects. The corn hybrid, DeKalb XL-45, was planted May 29, 1969, and Pioneer 3369A was planted May 8, 1970 and May 4, 1971. Average final stands were 43,000 plants per ha (17,400 plants per A) in 1969, 54,000 plants per ha (21,860 per A) in 1970, and 59,000 plants per ha (23,900 per A) in 1971. In 1970 corn was planted at a high rate and hand thinned to an exact population per row. Stand counts were taken in 1970 prior to thinning. In 1969 and in 1971 no hand thinning was done. Atrazine (Aatrex) and Lasso were used as broadcast pre-emergence herbicides. Cultivation was also used for weed control. Corn was planted in 76 cm (30 inch) rows in plots 18.3 m (60 feet) long and 4 corn rows wide. Hand harvest areas were 7.9 m (26 feet) long and the 2 center rows were used. Moisture percentages in the grain and tables for converting ear corn weights to shelled corn were used to calculate grain yields. Yields are reported on a 15.5% moisture in shelled corn basis.

Corn ear leaf samples were analyzed for plant nutrients by the Growth Sciences Center of the International Mineral Chemical Corporation, Libertyville, Illinois.

Results and Discussion

The use of the highest rate of urea (1,344 kg per ha or 1,200 lbs per A) in 1969, resulted in a decline of 0.7 pH unit in Fincastle soil and 0.5 pH unit in Chalmers soil (Table 1). In 1971, the soil pH was 5.3 on this treatment on the Fincastle soil. According to Barber (1), limestones having low percentage of particles passing through a 60-mesh sieve are not likely to be efficient in acting rapidly to correct soil acidity. With 36 to 38% passing a 60-mesh sieve, these limestones were apparently too coarse to completely correct acidity in time between application and pH measurement.

Soluble salts in both soils were higher as rates of nitrogen increased. This was true in both 1969 and in 1971 (Table 1). Soluble salts were not determined in 1970 when no growth irregularities were observed. In 1969 differences in numbers of plants per plot and yield related to treatment were observed (Table 2) even with 4.5 cm of rain in the 10 days following planting. In 1969 the stand (number of plants per plot) on the 1,344 kg per ha rate of nitrogen from urea was reduced to 33% and yields of corn grain were reduced to 37% of those which received 336 kg per ha of nitrogen. This reduction in stand cannot be attributed entirely to salt effect.

Hunter and Rosenau (2) found that in closed urea-soil systems, urea-soil mixtures out of contact with germinating corn seeds released gaseous ammonia into the atmosphere, and the ammonia inhibited germination and affected seedling development of corn. Similar effects were noted when corn was seeded in soils saturated with ammonium ion to the extent of 32% or more. The 1,336 kg per ha rate of N used herein would, if carefully mixed and all the ammonia (from urea) were absorbed on the exchange sites, saturate 80% of the cation exchange capacity of Fincastle soil and 40% of the exchange capacity of Chalmers soil. Highly significant reductions in stand were found at this rate of N on both soils. However, at the 672 kg per ha (600 lbs per A) rate of N, stand was reduced significantly only on the Fincastle soil. In all years N deficiency symptoms were observed on both soils in the no nitrogen treatment. In 1971, a year of high corn yields, these symptoms of N deficiency were severe. The significantly lower grain yield on both soils of the 672 kg per ha rate of N than the 1,344 kg per ha of N in 1970 was not thought to be meaningful because the 336 kg per ha treatment was also higher yielding. The general

TABLE 2. *Relation of rates of urea fertilizer application to plant population, leaf height, and yield of corn on two soils, 1969-1971.*

Kg/ha of N Applied Annually	1969		1970		1971	
	Plants per Plot (7.92 m x 1.52 m)	Grain Yield (kg/ha)	Maximum Leaf Height (6-26-70 cm)	Grain Yield (kg/ha)	Maximum Leaf Height (6-23-71 cm)	Grain Yield (kg/ha)
Fincastle silt loam						
0	61	7,500	81	4,450	113	1,470
168	63	8,540	100	8,950	115	9,240
336	65	8,140	97	8,840	116	10,020
672	48	6,120	99	8,220	91	8,490
1,344	21	3,020	100	9,010	92	9,250
LSD 05 for rates	10	1,120	14	680	18	1,610
Average	52	6,660	95	7,900	105	7,690
Chalmers silty clay loam						
0	59	7,500	114	6,379	140	2,990
168	51	9,350	128	9,615	181	11,880
336	59	9,040	138	9,697	181	12,450
672	55	8,710	143	9,044	175	12,720
1,334	31	5,310	148	9,941	165	12,690
LSD 05 for rates	9	1,690	10	730	7	2,110
Average	53	7,980	134	8,944	168	10,550
LSD 05 for soils	Not Sig.	yes ¹	yes ¹	yes ¹	yes ¹	yes

¹Yes means averages for the two soils are significantly different from each other at the stated or greater probability.

trend in yield was very slightly higher in rates above none. This trend was the result of heavier ears with the higher rates of N.

In 1971, even though grain yields were not reduced significantly, maximum leaf heights were reduced significantly at the two highest rates of N in the Fincastle soil and at the highest rate on Chalmers soil (Table 2). Total soluble salts and also nitrates were high in these two highest treatment rates of N from urea on Fincastle soil. Soluble salts were not as high on the Chalmers as on the Fincastle soil at the highest rate of N in 1971. High soluble salts in soil probably contributed to the reduced rate of growth of corn in the highest treatments on the Fincastle soil. Height of plants was reduced 20% on the Fincastle soil and to only 9% on the Chalmers soil in 1971. Germination and stand were not measurably affected by high rates of N, and neither were grain yields.

Plant analysis on corn ear leaves indicated that contents of manganese (Mn) increased from 4 to 15 times as rates of nitrogen were increased from 0 to 1,344 kg per ha (Table 3). This increased uptake of Mn was associated with a lower soil pH. Content of Mn in the ear leaves was 2 to 3 times as high from the 168 kg per ha rate of N on Fincastle soil as from the same rate of Chalmers soil. Soil Mn determinations were not made.

TABLE 3. *Relation of soil types and rates of nitrogen to manganese in corn ear leaves on two soils, 1970-71.*

Kg/ha of N Applied Annually	Part per Million of Manganese			
	Fincastle silt loam		Chalmers silty clay loam	
	1970	1971	1970	1971
0	35	33	14	<10
168	52	46	16	17
336	53	48	20	22
672	94	91	34	37
1,344	149	196	91	77
LSD 05	38	66	12	Not possible
LSD 01	64	93	19	Not possible

In conclusion, high rates of urea (672 or more kg per ha of N) incorporated into the plow layer of soil just before planting can reduce germination and yield of corn. Also, high rates of urea can cause soil pH to decline as much as 0.7 of one pH unit in one cropping season. After three applications of high rates of urea and two applications of limestone in three cropping years, soluble salts can become high enough to contribute to reduced maximum leaf height of corn in late June particularly on the low exchange capacity and low organic matter Fincastle soil.

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Weekly and Seasonal Changes in Total Suspended Particulate Concentrations at Indianapolis, Indiana¹

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Abstract

Daily total suspended particulate concentrations for the period of 1968-1970 at Indianapolis, Indiana, were analyzed to illustrate the weekly and seasonal variation in urban air pollution. Weekly and seasonal changes in air quality as measured by daily total particulate concentrations are identified; comparisons are made between the maximum monthly concentrations and the mean monthly frequency of stagnating anti-cyclones. Mean annual area concentrations for daily periods as measured by 19 monitoring stations in Indianapolis and Marion County, Indiana, are illustrated. Some reasons for the recorded changes in daily, weekly, and seasonal air quality in Indianapolis are proposed.

Introduction

It is generally understood that air pollution concentrations change from hour to hour, day to day, and from one season to another during the year. These variations have many causes, some of which are well understood. Extremely high levels of air pollutants are related primarily to very stable stagnating meteorological conditions for several days. Likewise, extremely low levels are related to very unstable meteorological conditions. But the normal hour to hour and day to day meteorological change, coupled with time and space changes in human activity in a given urban area, usually produces substantial periodic patterns in air pollution concentrations. These pattern changes must be properly assessed when planning a sampling procedure for the purpose of monitoring air pollution concentrations. Sampling at fixed times and places can produce highly biased data. It is for this reason that the authors took a rather critical look at periodic changes in air pollution concentrations of total suspended particulate over a 3-year period at Indianapolis, Indiana.

Data and Methods

Data used in this study consisted of 24-hour samples of total suspended particulate concentrations from 19 locations in Indianapolis, Indiana, from January 1, 1968, through December 31, 1970. These daily values were in micrograms per cubic meter ($\mu\text{g}/\text{m}^3$). The data

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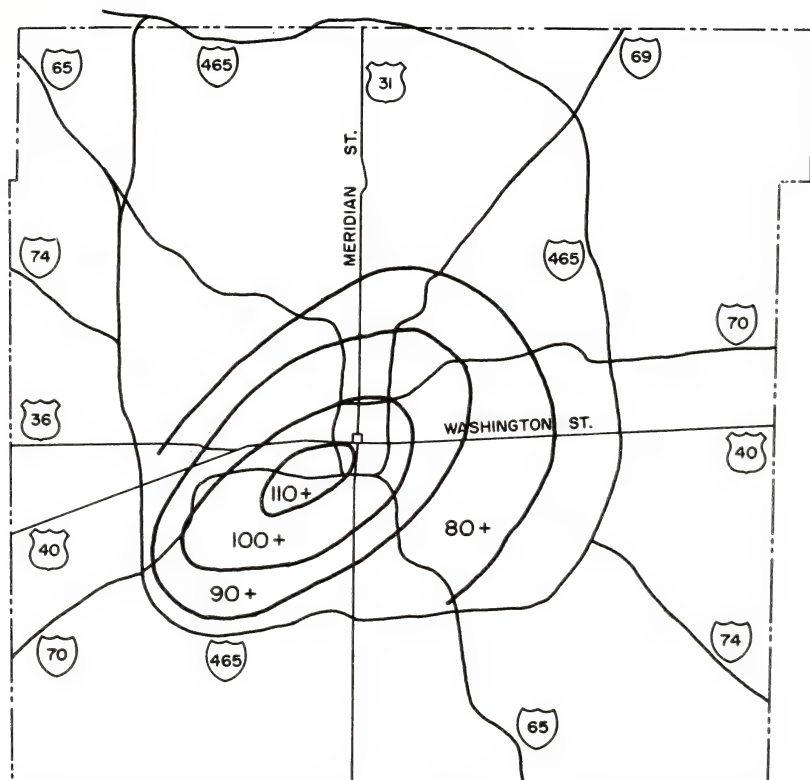


FIGURE 1. Mean daily total suspended particulate for Indianapolis, Indiana 1968-1970.

were recorded by the Environmental Control Division, Department of Public Works, City of Indianapolis.

Daily values representing a 24-hour period from 10 AM one day to 10 AM the following day from each of the 19 monitoring stations were averaged to produce the estimated isolines of mean daily total particulate reported in Figure 1. The total composite of daily values in consecutive 7-day weeks for the 3 years at Site 1 was 777 or 111 weekly periods. These daily values were further composited into 7-day period values for each day of the week, thus the mean value for each of the

TABLE 1. *The Analysis of variance for the composite of daily values for total particulate at Indianapolis, Indiana, 1968-70.*

Sources	D. F.	Sums of Squares	Mean Squares	F Ratio
Treatment	6	27565.0101	4594.1683	3.2704
Residual	766	1076051.2668	1404.7667	
Total	772	1103616.2768		

$F_{.05} = .210$

$F_{.01} = .280$

TABLE 2. *The analysis of variance for the composite of monthly values for total particulate at Indianapolis, Indiana 1968-70.*

Sources	D. D.	Sums of Squares	Mean Squares	F Ratio
Treatment	11	57549.0300	5231.7300	3.8060
Residual	761	1046067.2469	1374.5956	
Total	772	1103616.2768		

$F_{.05} = 1.75$

$F_{.01} = 2.18$

7 days in a weekly period is an average of 111 values. Similarly, all daily values falling within a given month were composited to produce monthly mean values. These data were subjected to an analysis of variance reported in Tables 1 and 2, and in Figures 2 and 3. Site 1 air sampling station is located near the City-County Building in downtown Indianapolis.

Results and Discussion

The National "Clean Air Act" passed by Congress in 1967 has many provisions, regulations, and suggestions as guidelines for achieving its goals. This Act clearly states that all areas within the United States shall come under the control of a State or Federal designated Air Quality Control Region. Further, the Act provides general guidelines and timetables for achieving compliance.

One of the goals as stated in guidelines, under the 1967 Clean Air Act, is that all areas in the United States should achieve an air quality, as measured by total suspended particulate matter, of $80 \mu\text{g}/\text{m}^3$ per 24 hours for an annual mean value.

With this in mind, the authors summarized 3 years of daily total suspended particulate samples collected within the City of Indianapolis and Marion County to produce Figure 1. This figure provides an estimated geographical picture of that area in Indianapolis where the air quality exceeded that annual mean value of $80 \mu\text{g}/\text{m}^3$ per day.

Once an air pollution problem area has been established, further monitoring must be continued to establish whether or not a geographical area has or has not achieved the goal of compliance for a given air pollutant. If one could assume that air pollution levels did not change much from hour to hour, day to day, and month to month, then only a few sampling days during the year would be adequate for establishing compliance within a given Air Quality Control Region. Secondly, if one could assume the air pollutants' changes in concentration were strictly random in time and space, then only enough samples to estimate the statistical error desired would be necessary. Unfortunately, neither of these two assumptions is likely to hold.

It is well known that air pollutants vary both in time and space. Over a yearly period or longer, variations in space are closely related to sources of pollutants in a given area. On the other hand, variations in time are closely related to changes in meteorological conditions and

the total activities of the community concerned. Since Figure 1 illustrates the mean estimated variation in space for the City of Indianapolis, the authors decided to subject the data to an analysis of variance in time at a given point. For this study, Site 1 was selected near the geographic center of Indianapolis. All daily values that were consecutive in 7-day runs over the 3-year period at Site 1 were subjected to this time-series analysis. The results of this effort are graphed in Figure 2, with the analysis of variance reported in Table 1.

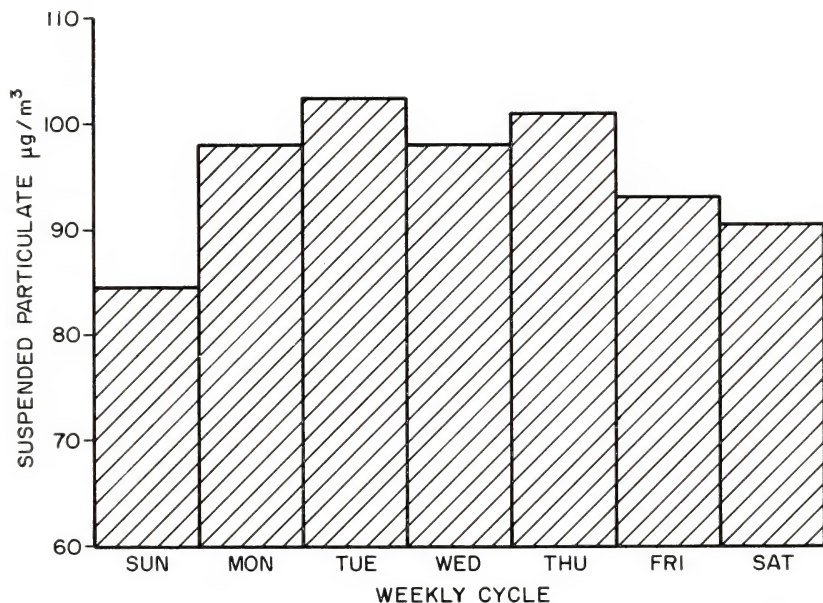


FIGURE 2. Mean daily values of total suspended particulate for each day in the week at Site 1, Indianapolis, Indiana, 1968-1970.

Figure 2 illustrates considerable change from day to day during the normal 7-day calendar week in mean daily total suspended particulate for Indianapolis. These mean changes from day to day during a weekly period are very significant as shown in Table 1. This analysis strongly suggests that taking air quality samples on one or two fixed days during a 7-day week is likely to produce biased data. The one day with the least bias in this analysis turns out to be Friday, since it is the mean daily value closest to the estimated weekly mean value.

A similar analysis was done for monthly periods. These results are reported in Figure 3 and Table 2. Here again, considerable variation exists in mean daily value of total particulate from one month to another and particularly between seasonal periods. The highest values occur during the summer season, particularly in August. The lowest seasonal values occurred in late autumn and early winter with the lowest in November.

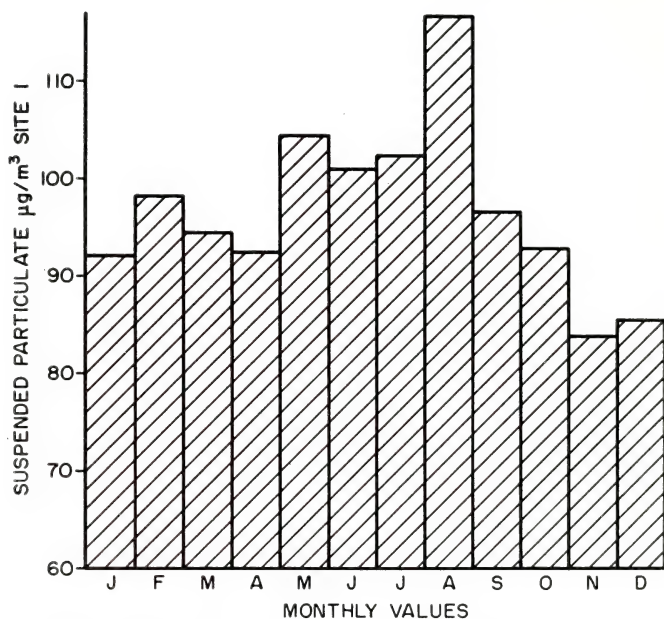


FIGURE 3. Mean monthly values of total suspended particulate for each month, at Site 1, Indianapolis, 1968-1970.

Variations in mean monthly values for daily total particulate at Site 1 in Indianapolis were subjected to further analysis as graphed in Figure 4. This graph reports the number of times per month that mean daily values exceeded one standard deviation above the monthly mean. Such above-normal values in air pollution concentration are

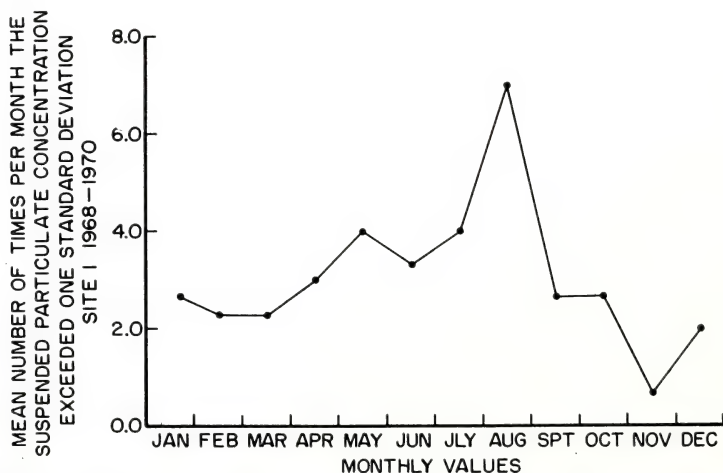


FIGURE 4. High air pollution occurrences at Site 1, Indianapolis, Indiana, 1968-1970.

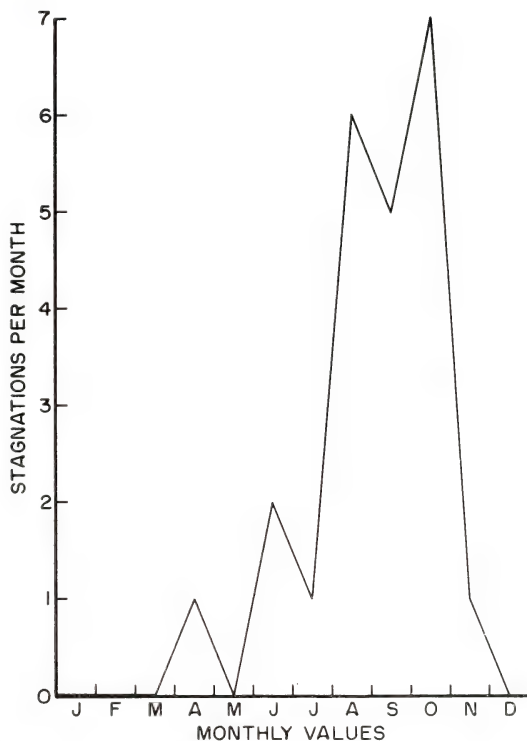


FIGURE 5. *Monthly number of stagnating anti-cyclones as reported by Korshover (1) at Indianapolis, Indiana, 1936-1965.*

usually associated with an extended period of extremely stable meteorological conditions. Therefore, it was decided to compare the results graphed in Figure 4 with the results reported by Korshover (1) on the mean number of cases per month of anti-cyclone stagnations in a 30-year period (1936-1965) for Indianapolis. Korshover's results are graphed in Figure 5. Note the unusually high number of cases in August, September, and October. Since Korshover's results represent a climatic norm, one can conclude that high levels of air pollution for extended daily periods should be expected during the late summer and early autumn at Indianapolis, Indiana.

This analysis shows that periodic sampling of air pollutants, as has often been the practice of air quality control districts, can result in seriously biased data. The reason for this possible bias is related to the fact that air pollution concentrations are subjected to periodic changes caused by the area patterns of total community activities and seasonal changes in meteorological conditions. Biased data from an air quality sampling network can be reduced by avoiding fixed sampling periods, such as one particular day per week or a particular set of days per month. Therefore, air quality samples should be taken in a manner

that minimizes the error in estimating the true mean for the period concerned.

It is generally accepted that air pollution concentrations vary from hour to hour during a normal 24-hour period. But since no hourly data existed at Indianapolis, the diurnal period could not be studied. Normally, the highest concentrations occur during the early morning hours of 7 to 9 AM, with the lowest values occurring between 1 and 4 PM.

Conclusions

From air quality data on total suspended particulate at Indianapolis, Indiana, from January 1, 1968, through December 31, 1970, it is concluded that day to day weekly variations and month to month seasonal variations are significant. Therefore, periodic sampling of air pollutants on fixed days of the week should not be practiced without proper correction procedures. Otherwise, seriously biased estimates in air quality can result.

Acknowledgements

The authors acknowledge Mr. Lewis F. Scott, Manager, and Mr. Wallace Benson, Chemist, of the Environmental Control Division, Department of Public Works, City of Indianapolis, for their many helpful discussions and suggestions concerning the data used in this study. The data were made available by the Environmental Control Division, Department of Public Works, City of Indianapolis.

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Temperature and Relative Humidity Inside Corn Canopies and in Standard Shelters during July and August, 1970 and 1971¹

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Abstract

Temperature and relative humidity within the microclimate of corn canopies were compared to macroclimate temperature and relative humidity recorded in a standard National Weather Service shelter for the period July 17 to August 15, 1970 and 1971. Unaspirated wet bulb and dry bulb thermocouples exposed at one meter were used to measure corn canopy temperature and relative humidity. A hygrothermograph was exposed in a shelter for macroclimate measurements. Corn canopy maximum temperatures were higher than shelter maximum temperatures and, conversely, corn canopy minimum temperatures were lower than shelter minimums during both years. Relative humidity within the corn canopy during this period was similar in both years although differences occurred in macroclimate relative humidity patterns.

This study suggests that temperatures obtained from standard National Weather Service shelters can be used to approximate temperatures within a corn canopy. Relative humidity, however, can be quite different within a corn canopy than within a standard shelter.

Introduction

Following any unfavorable agricultural situation such as delayed corn maturity in 1967 or the unprecedented development of Southern Corn Leaf Blight (SCLB) in 1970 and 1971 (3), agriculturists often look first to anomalous weather conditions for an explanation. Weather data are collected and published by the National Weather Service from a network of instruments exposed under standard conditions. These data furnish excellent macroclimatic comparisons, but unless relationships between these data and microclimatic conditions are known, a correct assessment of the effect of macroclimatic anomalies upon biological response within a micro-environment can only be hypothesized.

This paper describes temperature and relative humidity patterns observed within a corn canopy and compares them with those from standard instrumental exposures for a period from mid-July to mid-August in 1970 and 1971. These microenvironmental measurements were taken as part of a corn growth and development study conducted at the Purdue University Agronomy Farm, West Lafayette, Indiana.

Description of Experiment

A summary of published temperature data (4) for the 1970 and 1971 growing seasons and the 18-year (1953 to 1970) average for the Purdue University Agronomy Farm is shown in Table 1. In this study, a 30-day period from July 17 to August 15—a period important in the SCLB development—was examined during both years.

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TABLE 1. *West Lafayette 6NW, Purdue University, Agronomy Farm temperature summary °C.*

	April		May		June		July		August		September	
	Max ¹	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
Normal ²	16.1	4.6	21.8	9.7	27.0	14.7	29.2	16.7	28.2	15.4	25.2	11.2
1970	16.4	4.7	23.8	11.4	26.5	14.8	29.1	16.8	28.1	15.3	25.8	12.3
1971	16.7	2.4	20.6	7.8	29.8	17.3	27.3	15.6	27.2	14.2	26.1	14.2

¹Average daily maximum temperature and average daily minimum temperature, 8 AM observation day.

²1953-1970 average.

Environmental temperature and relative humidity data were from a hygrothermograph exposed in a medium National Weather Service shelter at the Purdue Agronomy Farm Agricultural Weather Station. The corn experimental area was located about 200 m north-northeast of the weather station. Corn population was about 62,000 plants per ha planted in 76 cm-wide rows oriented north to south. The corn canopies were approximately 2.7 m tall in both years with an average leaf area index during the study period of 3.5 in 1970 and 4.0 in 1971. Seventy-five per cent silk dates were August 3, 1970 and July 27, 1971.

Copper-constantan thermocouples (24-gauge wire) were utilized to measure dry and wet bulb temperatures at 1 m within the corn canopy. Dry bulb fluctuations were reduced by shielding the thermocouple junction with white tape. A wet bulb was formed by tying a muslin wick securely around a bare thermocouple junction and extending the muslin downward into a water reservoir. Output from the thermocouples was recorded on a multi-point recorder.

Relative humidities measured by wet and dry bulb thermocouples were compared with those from a hygrothermograph exposed at the same location within the corn canopy during September 1971. The hygrothermograph used in the corn canopy was calibrated with the shelter hygrothermograph before and after the September study. The least squares regression equation for the calibration period was $Y = 1.718 X - 70.266$, where Y is relative humidity taken from the hygrothermograph and X is that from the thermocouples. R^2 was 0.83. Since relative humidity in the shelter was recorded with a hygrothermograph, corn canopy relative humidities calculated from wet and dry bulb thermocouples data were corrected by this equation for this study.

Results and Discussion

Temperature

Temperatures in the shelter for the period from July 17 to August 15, 1970 and 1971 are shown in Figure 1. Daily maximum and minimum temperatures during 1970 were close to normal with periods both above and below normal. In 1971, daily maximum and minimum temperatures were well below normal with only a short period in August having above normal temperatures.

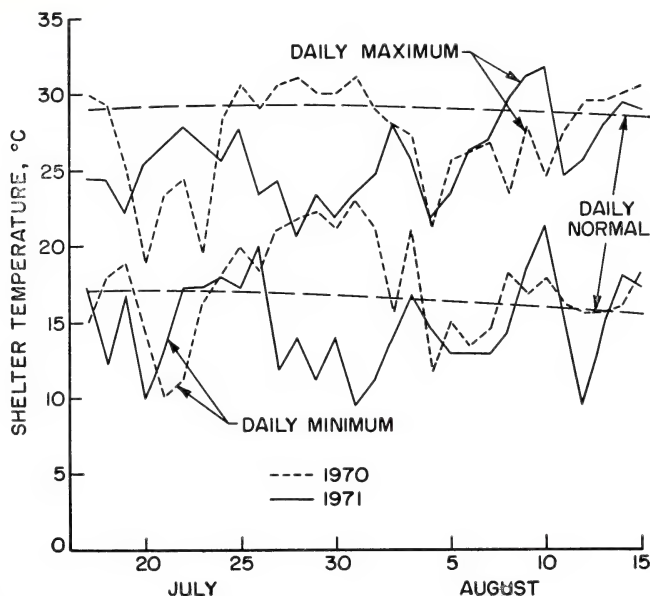


FIGURE 1. Daily maximum and minimum shelter temperatures at West Lafayette 6NW, Purdue University Agronomy Farm, July 17 to August 15, 1970, 1971, and 30-year (1931-1960) normal.

Hourly shelter temperatures averaged for the 30-day period from 17 July to 15 August show that 1971 temperatures were 2 to 3°C lower than 1970 temperatures (Fig. 2). An average hourly standard deviation of 1.3°C was found in 1970 and 0.9°C in 1971.

Differences between temperatures in the canopy and in the shelter for each hour are also shown in Figure 2. Nighttime temperatures within the canopy averaged 1.5°C lower than those in the shelter during 1970 and 0.5°C lower in 1971. Midday temperature differences reached a maximum of 1.2°C greater in the canopy than in the shelter during 1970 and 0.6°C during 1971. This difference between the two years can partially be explained by a denser canopy leaf area in 1971 than in 1970. The 1971 canopy did not permit as great a radiational exchange between the 1 m level in the canopy and the macroenvironment as did the 1970 canopy. Radiational heating and cooling of the canopy interior was therefore less during 1971 than during 1970. The early evening hours were a time of greatest temperature differentials between the macroenvironment (shelter) and the corn canopy. Rahn and Brown (2) have also found the corn canopy microenvironment to be warmer in daytime and cooler at night than the macroenvironment as measured in an instrument shelter.

Temperature differences in Figure 2 could reflect differences between exposures and also differences between types of instruments, *i.e.*, thermocouples within the canopy and a hygrothermograph in the shelter. The temperature data shown in Table 1 are from a third type

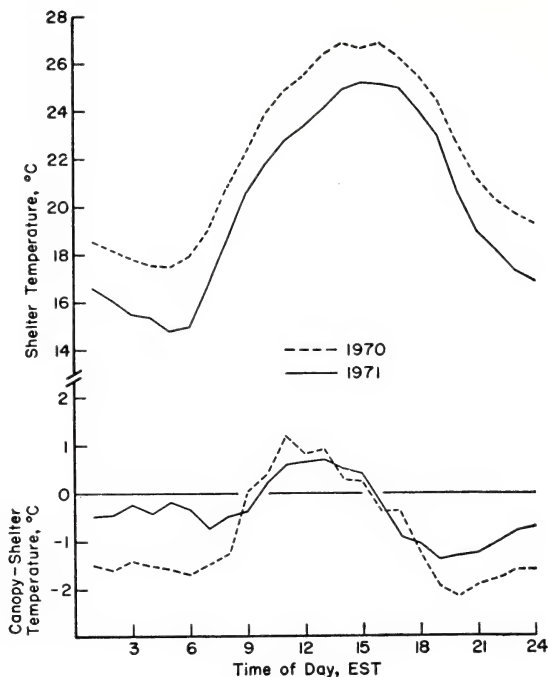


FIGURE 2. Average hourly shelter temperatures and average hourly difference, corn canopy minus shelter temperature, for the period 17 July to 15 August, 1970 and 1971.

of instrumentation, liquid-in-glass maximum and minimum thermometers. Accuracy in reading both time and temperature from hygrothermograms and thermocouple charts defeat quantification of any estimate of bias due to instrumental differences².

If temperature differences shown in Figure 2 are used to estimate maximum, minimum, or mean temperatures within a corn canopy from published climatological data, bias arising from the time of observation must be considered. Mitchell (1) has shown that estimates of mean temperature were near the true daily mean if the observational day ended at 8:00 AM, but the maximum and mean temperatures were biased upward if the observational time was 5:00 PM. An 8:00 AM observational day was used in this study for summarizing all temperature data.

²Published (4) maximum temperatures from liquid-in-glass thermometers averaged 0.6°C higher and minimum temperatures 0.7°C lower than those from a hygrothermograph exposed in the same standard shelter. A hygrothermograph and thermocouples exposed at 1 m within a corn canopy during September 1971, recorded the same nighttime minimum temperatures. Daytime maximum temperatures, however, were recorded about 1°C higher on the hygrothermograph. The higher daytime temperatures were due to sunfleck heating of the hygrothermograph while thermocouples remained in shaded locations during both the July-August and September periods.

Relative Humidity

Three basic instruments are used by the National Weather Service to measure relative humidity. Hygrothermographs, of the type exposed in a shelter for the data in this paper, depend upon absorption of water vapor by human hair. Hygrothermometers of the type currently exposed at airport weather stations consist of a ventilated dew cell to obtain dew point data. Relative humidity measurements with this instrument are usually lower than those recorded by a hygrothermograph. A third type instrument is the wet and dry bulb psychrometer. Although the psychrometer is usually force ventilated, an unspirated thermocouple version of this instrument was used for canopy measurement of relative humidity in this study.

Relative humidity data in the corn canopy were not recorded prior to July 26, 1970. Thus hourly relative humidities in the shelter and canopy were averaged for the 21-day period from July 26 to August 15 (Fig. 3). The 1970 hourly relative humidities in the shelter were generally higher than those in 1971. The largest differences, 5% to 10%, occurred during the morning hours. The 1970 and 1971 corn canopy relative humidities, however, were similar throughout the day.

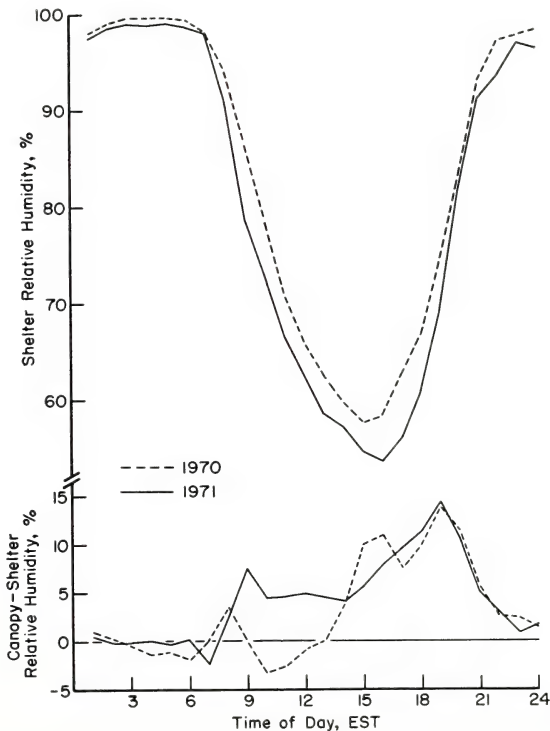


FIGURE 3. Average hourly shelter relative humidity and average hourly difference, corn canopy minus shelter relative humidity for the period July 26 to August 15 1970 and 1971.

Relative humidities in both the shelter and corn canopy were close to 100% during the nights in both years.

Hourly relative humidity differences, canopy minus shelter, were similar during the night, afternoon, and early evening hours in both years (Fig. 3). Morning relative humidities, however, did not show similar characteristics in 1970 and 1971. During 1970, relative humidity in the corn canopy and shelter changed at about the same rate until 2:00 PM, but in 1971 the shelter relative humidity decreased more rapidly in the morning than did the corn canopy relative humidity. Hourly standard deviations of the relative humidity differences were about 3% at night and 10% in the afternoon. The large afternoon deviations were caused by a few periods of rain and high humidity.

Conclusions

During the 30-day period, July 17 to August 15, 1970 and 1971, temperatures at 1 m within a corn canopy were lower at night and higher during the day than temperatures recorded in a standard National Weather Service shelter. The shelter relative humidity was greater in 1970 than in 1971 with the largest differences occurring during the morning hours. Relative humidities within the corn canopy, however, showed similar average hourly trends during the morning hours in 1970 and 1971. Relative humidity in both the corn canopy and shelter averaged close to 100% at night in both years.

These data suggest that temperatures obtained from standard National Weather Service shelters can be used to approximate temperatures within a corn canopy. Relative humidity, however, can be quite different within a corn canopy than within a standard shelter.

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Predicting Southern Corn Leaf Blight Development in 1971 by Computer Simulator EPIMAY¹

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Abstract

A disease simulator model was used on an experimental basis during 1971 to evaluate the effects of weather on the spread and multiplication of Southern Corn Leaf Blight. Weather data consisting of hourly temperature, humidity, wind speed, sunshine, leaf wetness and precipitation were used in the computer program to provide daily values for new infections, lesion accumulations and per cent leaf area affected. Results were then compared to observations provided through pathological reports, ground observer data and aerial photo-multispectral scanner information.. Weekly EPIMAY predictions compared favorably with the observed weekly change in blight. At stations where starting dates of EPIMAY and the initial observed infection were close, good comparisons resulted for per cent leaf area infected.

Introduction

During 1970 southern corn leaf blight, race T of *Helminthosporium maydis*, reduced corn yields in the United States by an estimated 10 to 15% according to crop reports of the U.S.D.A. Statistical Reporting Service. Losses were confined mainly to corn hybrids containing Texas male sterile-cytoplasm. The disease started in the Southern United States in May and June, then spread rapidly into the midwest corn belt during July and August (1). Some fields of corn in Indiana were completely destroyed by the epidemic.

Considerable effort was made during and following the 1970 growing season to establish the effect of weather on the development of the disease. EPIMAY, a disease simulator model, was developed by Dr. Waggoner and associates at the Connecticut Agricultural Experiment Station (2). The model was based on application of 1970 observed weather-disease information and laboratory tests. The results showed promise for application as an objective indicator of the favorability of weather for the development of southern corn leaf blight.

Barger (NOAA, EDS, Wash., D.C.) suggested national use of EPIMAY for the corn blight problem and in June 1971 a program, under the direction of Regional Climatologist, L. A. Joos (Joos, unpublished data), was established using computer facilities at the National

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Weather Service Central Region in Kansas City. The program was conducted over a 12-state area and included about 20 stations. To supplement and assist in evaluating the national program, an intensive application of the EPIMAY model was conducted at Purdue University for 8 stations in Indiana.

The Indiana program was a joint cooperative effort by research and operation groups at Purdue to identify and evaluate southern corn leaf blight by aircraft sensing and by collection of field observations, and thus testing the predictability of EPIMAY (using weather data).

In addition to airborne information, the Corn Blight Watch Experiment conducted by the USDA, NASA and Universities in seven Corn Belt States provided an organized collection of ground truth by extension agents and pathologists in a specific series of fields along the designated flight paths for sensing aircraft (Bauer, M., unpublished data).

Methods

The disease simulator model estimated the effects of temperature, humidity, wind, light and leaf wetness on all stages of the disease cycle. Other factors involved were effects of wind and rainfall on wash off, dry out and transport of spores. To determine the number of lesions formed, the model systematically considered the factors relating to spore production, dissemination and infection by the blight fungus. This was accomplished by a series of laboratory developed curves relating the single and combined effects of weather on the disease. Some modifications were made in the model by Purdue researchers to adjust for change in leaf area available for infection and to adjust starting dates to fit planting and various development stages of the corn crop (Peart, R., and O. Loewer, unpublished data).

Weather input to EPIMAY for the national and Indiana programs were provided by the daily reports from the Indiana agriculture weather observation network and by hourly teletype reports from the National Weather Service and Federal Aviation Agency teletype reporting stations. Data for each 3-hour period through the day, beginning at midnight Greenwich time (7 PM EST), were summarized, resulting in 8 data readings per 24-hour period from June 11 through September 2. Dew or leaf wetness were rated in five classes: no dew—zero duration, light dew—3-hour duration, moderate dew—6-hour duration, heavy—9-hour duration, and very heavy—12-hour duration. Duration of classes was determined through instrument measurements and visual checks. When dew was present hygrothermograph traces usually indicated 80 to 100% relative humidity. Light or brightness of the sky information was based on cloud cover from sunrise to sunset using 50% or greater cloudiness as a cloudy day. The data were punched on computer cards each Friday for processing by the LARS computer facility.

The computer output consisted of a daily listing of weather data including the starting, ending and inches of rainfall for each precipi-

tation period, leaf wetness as yes or no, and hourly rate of rainfall for each 3-hour period for checking the input data. Next came the results, a daily listing of new infections, new lesions, accumulated lesions and per cent of leaf area infected by lesions. Map summaries showing accumulated lesions, weekly increase and per cent leaf area infected at each location were then prepared for distribution to all Purdue groups and by telecopier to Climatology Research Laboratory, NOAA, Silver Springs, Maryland.

Results

EPIMAY estimated the number of blight lesions formed daily and provided accumulative totals for each station. Flat plateau-like portions of the curves (Fig. 1) indicate when weather was unfavorable for blight

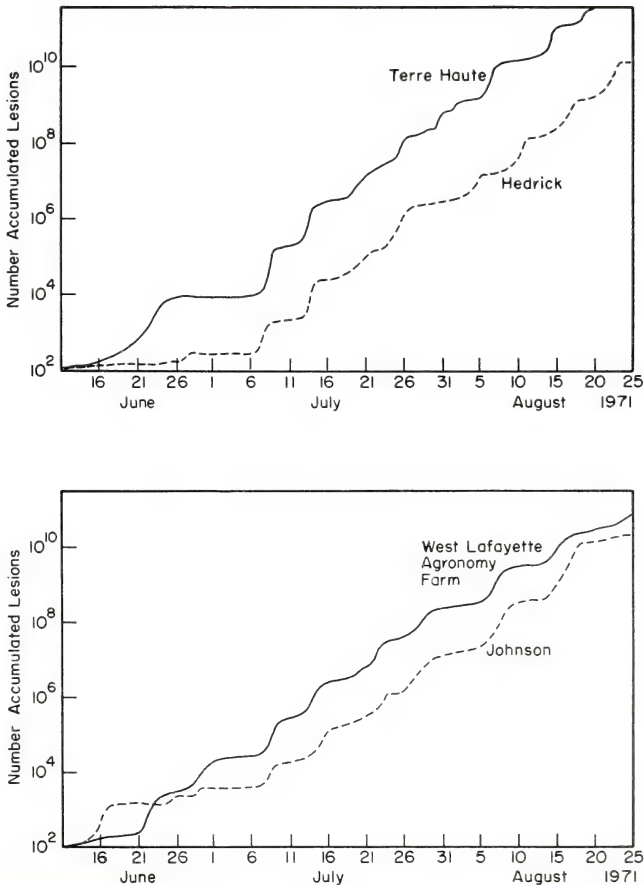


FIGURE 1. Accumulated southern corn leaf blight lesions during the 1971 growing season as predicted by EPIMAY for West Lafayette Agronomy Farm, Johnson, Hedrick and Terre Haute, Indiana.

development. During favorable periods sharp increases in the anticipated lesion numbers followed 72 hours after the onset of infection. This reflects the time interval from when fungus spores land on the corn plant until lesions become visible.

Curves showing accumulated lesions (Fig. 1) reached 10^8 values (level corresponding to approximately 20% leaf area coverage of lesions) 10 days earlier at West Lafayette Agronomy Farm and Terre Haute than at Johnson and 15 days earlier than at Hedrick. Accumulated

TABLE 1. *Comparison of EPIMAY predictions and field observations of per cent leaf area infected by southern corn leaf blight.*

Location	Starting Date 100 Lesions/ha)	Observation Date	Per cent of Leaf Area Infected	
			EPIMAY	Field Observations
Wanatah	6/21	7/31	2.5	0.5
Lafayette	6/11	7/26	8.5	1.0
Lafayette (Agron. Farm)	6/11	7/29	17.3	11.0 (Inoculated 7/1)
Hedrick	6/11	7/27	0.2	0.5
Indianapolis	6/11	7/28	0.5	32.0
Terre Haute	6/11	7/27	18.8	12.0
Vincennes	6/1	7/26	4.6	15.0
Evansville	6/1	7/27	9.8	8.0

lesion curves at all 8 stations showed similar slope but were displaced by 0 to 20 days for an equal level of lesions.

Per cent leaf area infected as predicted by EPIMAY, was compared to levels observed in corn fields of susceptible varieties (Table 1).

TABLE 2. *Weekly increase factors in number of lesions as predicted by EPIMAY.*

Factors in the Increase in Numbers of Lesion Over Number Previous Week							
Date	Wanatah	W.Laf.	Hendrick	T.Haute	Vincennes	Johnson	New Augusta
6/11-6/17	0	1	0	1	1	6	3
6/18-6/24	6	8	0	39	12	4	2
6/25-7/1	9	6	2	4	1	1	2
7/2-7/8	2	3	3	2	3	10	7
7/9-7/15	24	19	19	11	3	4	4
7/16-7/22	9	8	3	6	7	3	2
7/23-7/29	6	19	27	13	14	14	6
7/30-8/5	2	2	7	5	3	4	3
8/6-8/12	4	3	15	11	12	11	7
8/13-8/19	4	10	4	23	20	10	9
8/20-8/26	5	12	16	3	1	4	3
8/27-9/2	9	3	6	2	6	12	24

Reasonable agreement was found at 5 of the 8 locations where weather data and sample fields were near and quite poor agreement elsewhere.

Weekly increases in number of lesions were used to indicate the degree of favorability of weather for blight development (Table 2). Favorable weather produced 7 day lesion increases up to 39 times the number for the previous week.

Discussion

A number of limitations prevent conclusive evaluation of EPIMAY. In most cases, except at the Purdue Agronomy Farm, West Lafayette, and at Wanatah, weather stations were at distances of several miles to over 20 miles from the sampled corn fields. Variation from local influences of topography, soil moisture and rainfall patterns for these distances could account for some of the differences between observed and predicted disease levels (Table 1). A starting condition of 100 initial lesions per hectare were used for EPIMAY but large variation could occur in this level due to local inoculum sources.

It was also necessary to adjust EPIMAY to account for per cent of leaf area remaining available for new infections (Peart, R., and O. Loewer, unpublished data). Further adjustments of leaf area indices are needed to account for different planting dates and stages of development of corn.

Other factors contributing to variation in results that should be considered are the susceptibility of different corn hybrids and the general physical status of the crop from other diseases and insects. In 1971, early planted susceptible corn varieties matured far enough ahead of the late developing southern corn leaf blight to limit damage mainly to foliage and stalks.

The overall effects of the 1971 corn growing season also has some bearing on the 1971 southern corn leaf blight situation. Winter and early spring dryness and summer coolness were effective in reducing spore viability in the 1970 crop residue. No large scale influx of spores were noted by pathologists as in the previous year. Early planting plus a warm very rapid spring growth period caused corn development earlier than usual before blight fungus development. These plus other factors, such as reduced acreage of susceptible corn, were among the reasons for Indiana's improved blight picture.

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Some Effects of Soil and Ambient Air Temperature Differences on Tomato Growth¹

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Abstract

An experimental laboratory study was designed to evaluate the effect of temperature difference combinations on the growth response of tomato seedlings. Growth indices were changes in stem diameter, root-mass length, and stem height. The stem diameter was continuously monitored with a specially designed electronic micrometer.

An endogenous cyclic pattern of change in stem diameter was noted as a function of the light and dark intervals. Increases in root temperatures up to 35°C (95° Fahrenheit) for periods of approximately four days had at least temporary beneficial effects on growth. However, further increases in root temperature were deleterious to growth, particularly root development.

Introduction

One of the most troublesome environmental problems in the United States is thermal pollution in water. It is readily apparent, due to the importance of temperature on metabolic and respiratory processes of living organisms, that release of large volumes of heated water to waterways and lakes can be an upsetting influence on natural life cycles. Heated waste water, because of its low grade temperature condition, has little industrial value and controls are both costly and complicated. Mechanical cooling towers and cooling ponds are the most common methods of controlling waste heat output but both are rather inefficient with little capital return accruing to their use. A possible alternative would be to find beneficial uses of the heated waste water.

One proposed solution is to use the heated water for irrigation and environmental control of crops. However, depending on the means of application of this water to plants, relatively large temperature gradients between plant tops and the root systems could exist.

The purpose of the reported study was to evaluate some of the effects of soil and ambient air temperature differences on the growth response of tomato seedlings which might occur as a result of using thermally polluted water as the source of water for irrigation (1).

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Literature

Plant growth may be defined as cell multiplication with or without an increase in volume or an increase in volume without cell multiplication. In the reported study only non-destructive measurements of volume were used as indices of plant growth. Measurement of stem diameter was after Splinter (5) who reported on the design of an electronic micrometer using a linear variable differential transformer to give continuous monitoring of plant stem diameter. Using this device, he also showed a high correlation coefficient between stem diameter and total leaf area for plant species exhibiting apical dominance.

The effect of temperature gradients between plant tops and their root systems has been previously investigated (4) but more so in recent years (3, 6) because of the possibility of thermally polluted soil water and because of improvements in instrumentation and experimental control. Currently, Boersma from Oregon State University is studying the effects of heating soil under field conditions by using buried electrical cables to simulate irrigating with thermally polluted water (2).

Equipment and Procedure

Three-week old tomato seedlings (Heinz 1350) were "transplanted" from a gravel to a mist culture. Transplanting was accomplished by securing a plant stem in the top side of a root environmental chamber with a cork and floral putty so that the root system would extend into the chamber. The root environmental chamber was in turn contained within a larger growth chamber as shown in Figure 1.

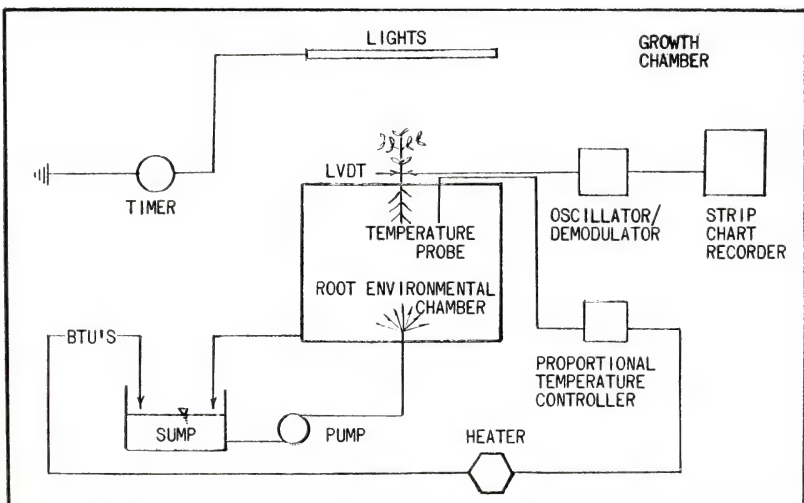


FIGURE 1. *Experimental setup showing the root environmental chamber within the larger growth chamber.*

Nine different root-top temperature combinations were used. Ambient air temperatures of 20 and 25°C were combined with 5°C increments of "soil" temperatures ranging from that of the ambient air to 40°C.

In the root environmental chamber, the plant roots were continuously sprayed with modified Hoagland's #1 nutrient solution. The temperature of the solution was controlled by a temperature probe, a proportional temperature controller, and a heater to $\pm 0.25^\circ\text{C}$ once equilibrium conditions were established. The root environmental chamber, which was transparent, was covered to provide a darkened space for root growth.

In the growth chamber, the air temperature was controlled at either 20 or 25°C $\pm 0.3^\circ\text{C}$, the relative humidity at $70 \pm 2\%$, and the light intensity at 1200 ± 100 ft-c. The day was divided into 16-hour continuous light and 8-hour continuous dark periods.

The growth response of the tomato seedlings was evaluated from changes in stem diameter which were continuously monitored with an electronic micrometer and from changes in root-mass length and stem height over the time period for each experiment. Each experiment lasted approximately 4 days. For any one experiment only the stem diameter of one tomato plant was measured. The equipment used to measure stem diameter consisted of an LVDT (linear variable differential transformer) head assembly, an oscillator/demodulator (Automatic Timing & Controls, Inc., Series 6101E) and a potentiometric strip chart recorder. This equipment is schematically represented in Figure 1 and the LVDT head assembly in position for monitoring change in stem diameter is shown in Figure 2.

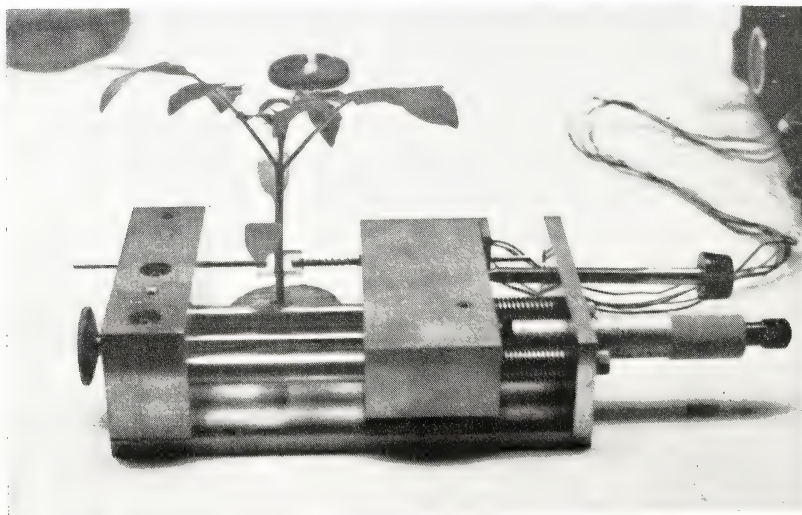


FIGURE 2. LVDT head assembly positioned for monitoring change in stem diameter of a tomato plant.

The LVDT (ATC No. 6233A) was enclosed in a precisely machined central housing. The head assembly was constructed of non-magnetic stainless steel and Plexiglas to prevent stray electrical current interference with the LVDT. A micrometer head (Starret Co. T263RL) was used to provide precise mechanical positioning of the LVDT armature in the center or null position of the coil.

The LVDT head assembly provided a non-destructive means of measuring stem diameter. The only back pressure, considered negligible, was caused by a small coil spring which was used to keep the LVDT in constant contact with the plant stem. When operating, the oscillator/demodulator was used to excite the LVDT primary coil, amplify the a-c output with phase sensitivity and convert it to a d-c signal suitable for direct readout on the strip chart recorder. The readout was thus directly proportional to the differential transformer armature displacement. Readings of 10^{-3} mm were considered accurate without readout interpolation.

Results and Discussion

The growth response of a tomato plant with leaf temperatures of 20 and 25°C, respectively, and root temperatures ranging from 20 to 40°C are shown in Figures 3 and 4. These curves were smoothed through many data points which also exhibited diurnal fluctuations. At a 40°C root temperature for both leaf temperatures, increases in stem diameter did not occur after 72 hours. Prior to this time, the roots of

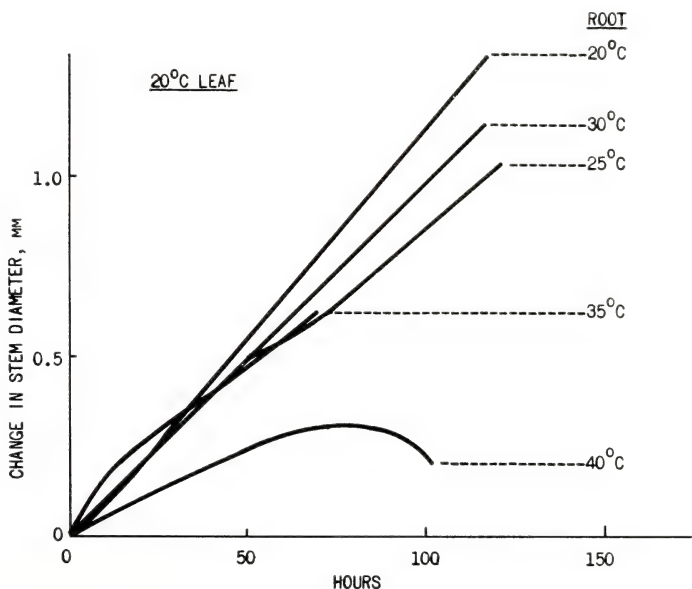


FIGURE 3. Growth response of tomato plants with 20°C leaf temperature and root temperatures ranging from 20 to 40°C.

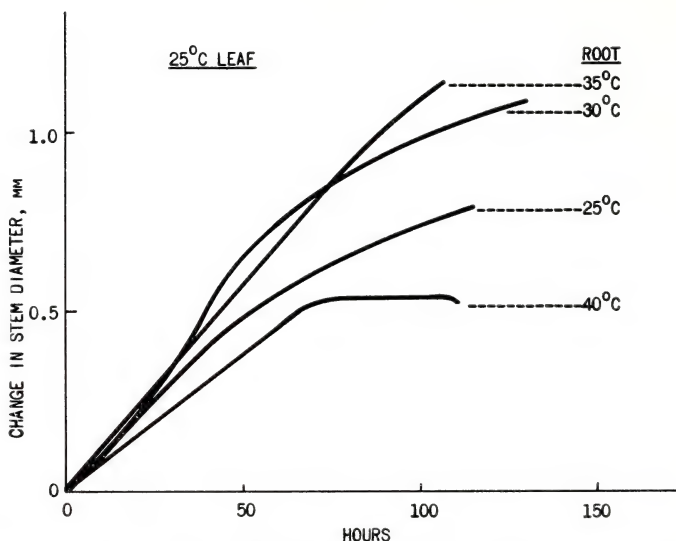


FIGURE 4. Growth response of tomato plants with 25°C leaf temperature and root temperatures ranging from 25 to 40°C.

the tomato plants showed little secondary root growth and were much darker in color than had been observed with any of the lower root temperatures. However, little visual damage to the leaf and stem portions of the plants was noted up to 72 hours but by 96 hours severe wilting and obvious death had occurred.

For all root temperatures less than 40°C, the stem diameter increased throughout the length of the experiment for both leaf temperatures. Trends if any between these root temperatures were not discernible for a given leaf temperature. However, increases in stem diameter were more linear for the 20°C than for the 25°C temperature during this stage of plant growth.

Effects of root temperature on the growth rate of tomato plants as measured by stem and root-mass lengths are shown in Figures 5 and 6. Both the stem and root-mass lengths are the average of three tomato plants for any one experiment. The maximum growth rate as measured by stem length occurred at a 30°C root temperature for both the 20 and 25°C leaf temperatures. The growth rate for the 25°C leaf temperature, however, was about double that of the 20°C leaf temperature. The maximum growth rate as measured by root-mass length occurred when the root temperature was 5°C higher than the leaf temperature. The 25°C leaf temperature condition produced about 1.5 times the growth rate of the 20°C leaf temperature when root-mass length was used as the growth index. The optimum temperatures for obtaining maximum stem and root-mass growth in these experiments were 25°C leaf and 30°C root temperatures.

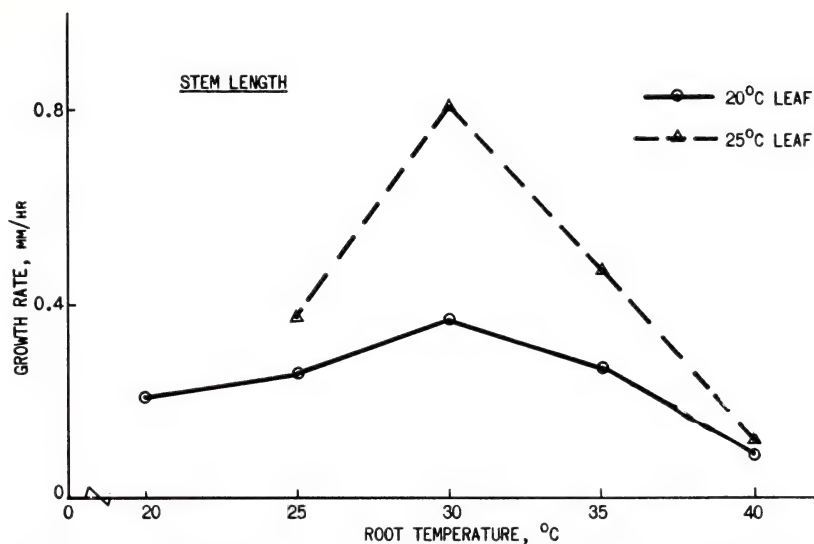


FIGURE 5. Effect of root temperature on the growth rate of tomato plants as measured by stem length.

In all experiments, the diameter of the plant stem changed with respect to the light and dark periods. Figure 7 is an expanded example of the diurnal growth response of a tomato plant with 20°C leaf and 20°C root temperatures. The growth rate based on change in stem

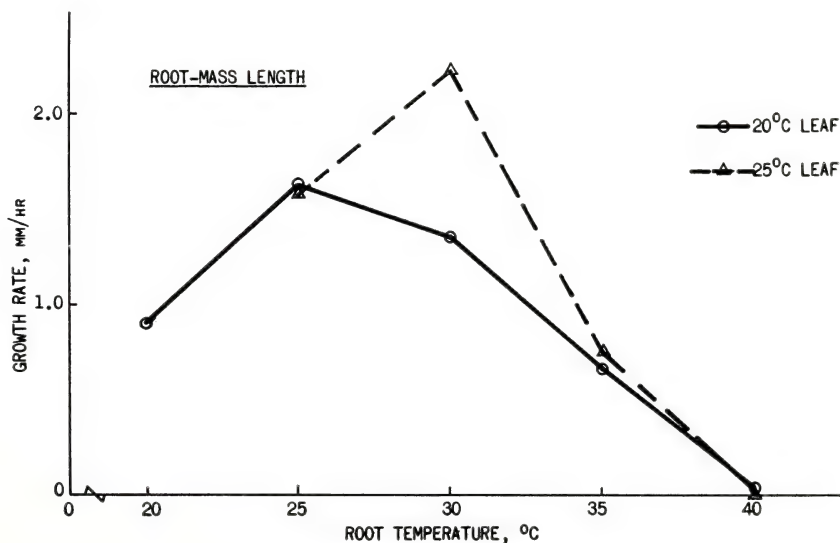


FIGURE 6. Effect of root temperature on the growth rate of tomato plants as measured by root-mass length.

diameter tended to decrease over the latter portions of both the light and dark intervals. This condition may have been caused by "fatigue" during the light periods and consumption of photosynthetic products during the dark periods. A rapid increase in stem diameter always occurred shortly after the climate chamber was darkened. This rapid increase in growth was probably caused by a change in the water regime within the plant. Turgor pressures tended to increase rapidly because of the closing of the stomata in the absence of light and because of the probable cooling of the leaves which would cause the energy available for transpiration to decrease.

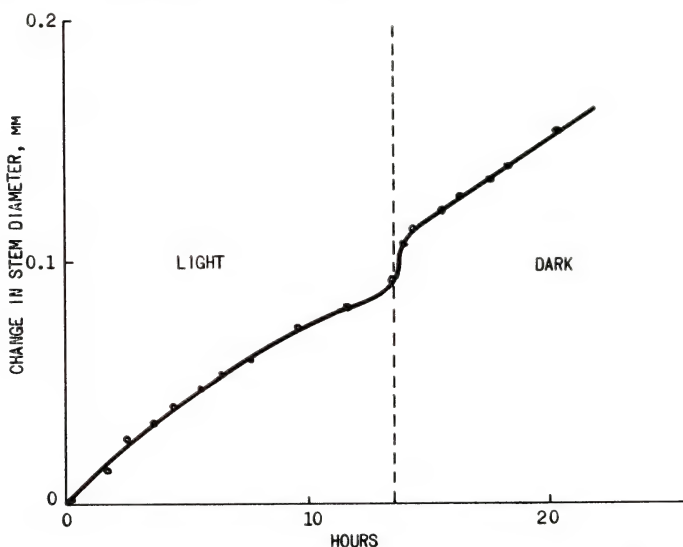


FIGURE 7. Diurnal growth response of a tomato plant with 20°C leaf and 20°C root temperatures.

Conclusions

Based on this study, the optimum temperatures for this stage of tomato plant development appear to be 25°C (77°F) leaf and 30°C (86°F) root temperatures. At these temperatures, the rate of growth as measured by increases in stem length and root-mass length was maximum. The rate of growth as measured by increases in stem diameter, although not always maximum under these temperature conditions, was always high. A continuation of these optimal conditions might be expected to result in maximum fruiting but this is only conjectural.

The LVDT head assembly provided a versatile and highly precise means of measuring changes in stem diameter in a non-destructive manner. Its precision was illustrated in the manner by which it was able to measure the small cyclical changes occurring between light and dark periods.

At 40°C (104°F) root temperature, the tomato plants died. The deleterious effect of this temperature was quickly noticed by the condition of the root system but approximately 3 days passed before it became noticeable from measurements of stem diameter. For lower temperatures, growth trends as measured by changes in stem diameter were not discernible. At this particular stage of tomato plant growth, measurement of changes in stem diameter should be coordinated with other growth indices and visual inspection to give much information concerning overall plant response. However, each type of measurement can yield useful information about certain physiological responses of the plant.

Heated waste water usually varies anywhere from 25 (77°F) to 50°C (122°F). Over the first half of this temperature range, direct application of the heated waste water to the soil would probably give beneficial growth results. However, over the last half of this temperature range, some cooling of the water should be accomplished during the application process. Under these conditions, sprinkler irrigation should be considered because the spray drops tend toward equilibrium with the wet-bulb temperature. The major objection to the use of heated waste water by tomato plants or for that matter any plant is not whether it can be beneficially used—it can—but the fact that it may be largely a consumptive use. Over-irrigation and the collection of return flow for reuse could reduce the percentage of applied water lost to consumptive use, however.

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ZOOLOGY

Chairman: WILLIAM J. BRETT, Department of Life Sciences
Indiana State University, Terre Haute, Indiana 47809

DOROTHY ADALIS, Department of Biology, Ball State University
Muncie, Indiana 47306, was elected Chairman for 1972

ABSTRACTS

Perception of the Plane of Polarized Light and Its Use by Orienting Salamanders.¹ KRAIG ADLER, Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556, and DOUGLAS H. TAYLOR, Department of Zoology, Miami University, Oxford, Ohio 45056.—Tiger salamanders (*Ambystoma tigrinum*) were trained to move along a specific axis in an indoor tank under a polarized light (produced by a polarizing filter, HN-38 Bausch & Lomb). Orientation tests were performed indoors in a small water-filled arena which was completely enclosed in opaque plastic. Animals were placed individually in the center of the arena beneath the polarized light source, then released and allowed to move to the edge of the arena where they were scored. Directional responses of salamanders in the arena were the same with respect to the plane of polarization as they had been in training. Rotation of the polarization filter by 90° produced corresponding changes in the direction of movement.

Orientation to the plane of polarization can be demonstrated in sighted and blinded animals. However, if opaque plastic is inserted over the skull of blinded animals, movement is at random; oriented movement is restored, however, when clear plastic is substituted in these animals. These studies suggest that the critical receptor for polarized light is extraoptic.

A Population Study of the Ozark Hellbender Salamander, *Cryptobranchus alleganiensis bishopi*. CHARLES E. MAYS, Department of Zoology, DePauw University, Greencastle, Indiana 46135, and MAX A. NICKERSON, Department of Zoology, Arkansas State University, State University, Arkansas.—During the summers of 1969 and 1970, an extensive mark and recapture study of *Cryptobranchus alleganiensis bishopi* was made on the North Fork River, a branch of the White River, Ozark, Co., Missouri. It was estimated that there are 1,142 salamanders in a 1.65 mile study area ($N = 439$). From length and weight measurements, it appears that the population consists of at least four age groups. According to a biomass estimate, there are 919 pounds of *C. alleganiensis bishopi* in this area. One riffle has a density estimate of one hellbender per 182 square feet.

¹Supported by an Indiana Academy of Science Grant, NSF GB-30647, and NIH FR 07033-05 to Adler; NSF Post-doctoral Fellowship GU-2058 to Taylor.

Observation of feeding activities as well as stomach analysis indicates that crayfish are the principal food source of *C. alleganiensis bishopi*. Cannibalism and egg-eating, which have been observed in the laboratory, may be important factors in maintaining population stability.

Specific Estrogen Binding Sites in the Nuclear Fraction of the Rat Uterus. J. H. CLARK, J. A. ANDERSON, and E. J. PECK, JR., Department of Biological Sciences, Purdue University, Lafayette, Indiana 47907.—Numerous investigations have demonstrated that the interaction of estradiol -17 β with the rat uterus is probably a two-step process in which the estrogen first binds with a cytoplasmic component to form a complex which subsequently moves to the nucleus. A method has been developed for the determination of the number of these nuclear binding sites in estrogen sensitive tissues. The method is based on the observation that estradiol, previously complexed with nuclear binding sites as a result of hormonal injection or ovarian secretions, is freely exchangeable with ^3H -estradiol during *in vitro* incubations of the nuclear fraction. This technique has revealed that the injection of estradiol -17 β results in an increased number of nuclear receptors in the uterus, vagina and anterior pituitary but has no effect on kidney or muscle. In addition, the injection of estrogenic hormones, but not testosterone or progesterone results in increased quantities of nuclear binding sites in the immature uterus. Fluctuations in the quantities of nuclear binding of the uterus as a function of endogenous hormonal levels were also examined. In mature cycling rats, uterine nuclear receptor concentrations were: proestrus, 2.44; estrus, 0.58; metestrus, 0.42; and diestrus 1.42 pico moles/per milligram DNA. These data reveal a cyclic fluctuation of nuclear receptor concentration which parallels the ovarian estrogen secretory rate during the estrous cycle.

Effects of Differentiated Brain on the Development of the Nervous System of the Explanted Chick Embryo. ABDULLA LAIRJE and NORMAN A. DIAL, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 42809.—One theory of development suggests that differentiated tissue inhibits in some way the differentiation of the same type of tissue in the early embryo. Blastoderms of chick embryos incubated for 24 hours were explanted to an agar-albumin-glucose medium made up in Howard Ringer solution and further incubated in Petri dish moist chambers for a period of 12 to 24 hours. Small pieces of brain from 7-10 day incubated embryos were placed on the blastoderm near the developing brain of the explant or in small depressions in the medium under the anterior portion of the explant. Homogenized brain mixed in the medium was also used. As a control, chick leg muscle from 7-10 day incubated embryos was used in the same manner. Untreated controls were also used. Fifty-seven (40.7 per cent) of 140 explants treated with differentiated brain showed brain abnormalities, while 15 of 74 (20.3 per cent) muscle-treated controls showed such abnormalities. Twenty of 112 (17.9 per cent) untreated controls had brain abnormalities. The usual kinds of abnormal changes observed included rudimentary or absent optic vesicles, reduced size

and rounded or pear-shaped anterior neural structures. These morphological changes were limited primarily to the forebrain.

Coccidial Immunity Studies In The Grey Squirrel¹ THOMAS JOSEPH, Department of Biology, Indiana University at South Bend, South Bend, Indiana 46615.—The response of the grey squirrel *Sciurus carolinensis* to infection with *Eimeria lancasterensis* and *E. confusa* was investigated. All 17 squirrels live-trapped from Tippecanoe County, Indiana, were naturally infected with *E. lancasterensis*. One of the squirrels carried a mixed infection with both parasites, but *E. confusa* disappeared after a week in captivity. The infection with *E. lancasterensis* persisted throughout captivity in all squirrels. This parasite infected the epithelial cells of the distal two-thirds of the villi. When experimentally inoculated with *E. confusa*, the squirrels took the infection with a patent period that ranged from 7-15 days. The patent period was shortened by repeating the infection; however, additional infections proved to be negative. *Eimeria confusa* infected the entire villus and penetrated deeper into the epithelial cells. The results of the study lead to the following tentative conclusions: 1) Grey squirrels do not develop immunity to *E. lancasterensis*, but will become immune to *E. confusa* and remain so for a period greater than 5 months; 2) Repeated infections with an interval of 1 month do not produce immunity; 3) It appears that immunity is more likely to develop against coccidia that penetrate deeper into the host tissues and may be a factor responsible for the low incidence of *E. confusa* in nature.

¹Supported by a grant from the Office of Research and Advanced Studies, Indiana University.

Relationship between Metabolic and Emergency Rhythms in *Drosophila melanogaster*. KAREN BELCHER, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—Oxygen consumption and lactic dehydrogenase (LDH) activity were measured to determine a metabolic rhythm and this rhythm was then compared with the emergency rhythm and the relationship determined. Oxygen consumption was determined for *Drosophila melanogaster* pupae that had been exposed to 12:12 or 1:23 light-dark (LD) regime and LDH activity was determined for pupae exposed to 12:12 LD regime.

Bimodal circadian fluctuations of oxygen consumption were found to exist in pupae of *D. melanogaster* exposed to 12:12 or 1:23 LD regime. Pupae under both regimes appeared to demonstrate an anticipatory change in oxygen consumption associated with the change in illumination. Techniques employed in the study did not permit detection of any regular fluctuations in LDH activity but a steady decrease in LDH activity during pupal development was evidenced.

The oxygen-consumption trend for the entire pupal stage showed a high at the time of emergence, but the diurnal rhythm for oxygen consumption showed a low at the time of emergence. These data suggest that emergence occurs at a metabolic low in the diurnal cycle.

Emergence data for the population showed an anticipatory change in the level of emergence to a change in illumination. A dark to light change was accompanied by an increase in number of emergents and a decrease in oxygen consumption. Emergence maximum showed a 3-hour lead over the oxygen-consumption maximum. Changing the LD regime from 12:12 to 1:23 and changing the time of the 1 hour of light by 12 hours in the 1:23 LD regime produced similar changes in the phasing of both oxygen-consumption and emergence rhythms.

Sites of Cell Proliferation in a Colonial Hydroid, *Campanularia flexuosa*. ROBERT L. SUDDITH, Department of Zoology, Indiana University, Bloomington, 47401.—The colonial hydroids exhibit diverse and specific patterns which are generated during the normal growth of the colony. Analysis of these growth patterns requires an understanding of the mode of growth. Morphological growth is expressed in the colonial hydroids as terminal elongation concomitant with an increase in colony mass (all hydroids) and pedicels (thecate hydroids). The sources of growth—primarily cell proliferation and secondarily increase in cell mass—have been thought to reside in the site of morphological elongation, the stolon tip, in a situation analogous to an apical meristem in plants.

When bands of cells in an actively growing stolon are marked with Nile blue sulfate, their movement does not correspond with that to be expected if cell proliferation is in the terminal region. We have undertaken a series of pulse-chase experiments using ^3H -thymidine as a marker to determine the site(s) of cell proliferation in an elongating stolon. Colonies were placed in filtered sea water containing ^3H -thymidine at a concentration of 5 micro Curie per milliliter for 1 hour and then incubated in filtered sea water containing 10^{-4} molar cold thymidine for one hour, before fixation. The tissue was fixed in Bouin's, embedded in Paraplast, and sectioned at 6 microns. The slides were coated with Kodak NTB-3 liquid nuclear tracking emulsion and exposed for 29 days. The developed slides were stained with Harris hematoxyline and counterstained with eosin.

Detailed analysis of the position of labeled cells following the 1 hour pulse with ^3H -thymidine reveals that only a few cells are labeled in the terminal 200 microns of the stolon tip. In the region 0.5-1.0 millimeter behind the tip there is a high density of labeled cells; beyond this to a distance 2.4 millimeter behind the tip there are few labeled cells. This is in direct contrast to the concept of a restricted terminal cell proliferation region. Proliferation of cells elsewhere than at the stolon tip is compatible with the results of vital staining experiments of Hale, Crowell, Syttenbach, and Suddith. In the particular stolon studied, the region containing a high density of labeled cells is at the level at which a new hydranth would be expected to develop.

Some Effects of Methylmercury on Early Frog Development. NORMAN A. DIAL, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—Little is known of the effects of methylmercury on early embryonic development. Preliminary investiga-

tions utilizing embryos of the frog (*Rana pipiens*) have been conducted. Embryos in various stages of early development were treated with various concentrations of methyl mercuric chloride in Holtfreters growing solution. The extremes in concentration ranged from 0.2 to 200 parts per billion (ppb). Solutions were changed daily to maintain a relatively constant exposure to a given concentration. Duration of exposure ranged from 2 days at higher concentrations to 7 days at lower concentrations.

Results indicated that selective developmental defects (poor tail development) may occur at concentrations as low as 1-5 ppb when treatment was begun in the late blastula and neural plate stages. Concentrations of 80 ppb or more inhibited development altogether within 36 to 48 hours when treatment was begun in early cleavage and late neural plate stages. A graded response as a result of time and concentration was evident. For example, midgastrula eggs treated with 10 ppb methyl mercuric chloride resulted in stunted, irregular tails and a slight slowing of body development after 6 days of exposure. On the other hand, eggs treated at the 32-cell stage of cleavage with 20, 40, 60, 80 and 100 ppb methyl mercuric chloride showed the following after 24 hours: Controls—late blastula—early gastrula; 20 ppb—same as controls; 40 ppb—same as controls; 60 ppb—mid-blastula; 80 ppb—mid-blastula; 700 ppb early blastula. After 48 hours controls were in the neural plate stage, while the 20, 40, 60, 80 and 100 ppb treated groups showed the following: Complete dorsal lip, early dorsal lip, late blastula, mid-blastula and early blastula, respectively. No further development occurred in any of the treated groups while controls continued to develop normally. Further investigation is continuing to determine uptake, localization and whether or not stage tolerance exists.

NOTE

Fish Populations in the White River near Petersburg, Indiana.

ROBERT S. BENDA¹, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—A 6-year study of the fish populations in the White River near Petersburg, Indiana, was concluded in October 1970. Two hundred and twenty-seven collections were made in a total of 237 hours. Fifty-three and one-half hours were used in 63 seining collections, 44 1/2 hours were used in 111 AC electro-fishing collections, and 139 hours were used in 53 DC electro-fishing collections. Collections were made in all habitats during the months of May through November each year.

The most frequently captured fish, based upon the per cent of collections containing at least one specimen, are as follows:

Gizzard shad, *Dorosoma cepedianum* (69%); Carp, *Cyprinus carpio* (44%); River carpsucker, *Carpionodes carpio* (37%); Spotted bass, *Micropterus punctulatus* (34%); Longear sunfish, *Lepomis megalotis* (34%); Emerald shiner, *Notropis atherinoides* (34%); Spotfin shiner *N. spilopterus* (31%); Bullhead minnow, *Pimephales vigilax* (31%); Longnose gar, *Lepisosteus osseus* (23%); Bluegill, *Lepomis macrochirus* (22%); Silvery minnow, *Hybognathus nuchalis* (20%); Freshwater drum

Aplodinotus grunniens (18%); Shortnose gar, *Lepisosteus platostomus* (15%); Sand shiner, *Notropis stramineus* (14%); Steelcolor shiner, *N. whipplei* (13%); river shiner, *N. blennioides* (13%); Green sunfish, *Lepomis cyanellus* (12%); Orange-spotted sunfish, *Lepomis humilis* (11%); Black crappie, *Pomoxis nigromaculatus* (11%); and the white crappie, *Pomoxis annularis* (11%). The other 49 species were collected less frequently and can be found listed in two other works (1, 2).

The Mosquitofish, *Gambusia affinis*, was captured twice. A single specimen was collected in a slough area in 1969 and one specimen was collected in the main river in 1970. This is the farthest north this species has been collected in Indiana according to published records (3, 4).

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Primary Social Relationships and Cattle Behavior¹

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Abstract

Holstein and Holstein-Red Danish dairy calves were removed from their mothers within 30 minutes after birth and before nursing. Thirty-six calves were raised from birth until 18 weeks of age on 1 of 4 treatments: Group SS, fed individually-penned individually; Group ST, fed individually-penned in groups of three; Group TS, fed in group of three-penned individually; and Group TT, fed in groups of three-penned in groups of three. Animals were then maintained in one group on pasture until their first parturition. Subjects raised together were more vocal, ignored and did not protect their young, partially cleaned them and failed to allow nursing. Raised separately cows had few vocalizations, tended to fully clean, protected their calves and allowed nursing. At second calving all cows cleaned, nursed and protected their young. Those showing poor maternal behavior at first calving remained less efficient at second calving. There were no significant differences among the four groups as measured by ease of training to enter the milking parlor, frequency of kicking, temperament and milking ease.

SS Holsteins ($P < .05$) produced more milk than those fed and raised under other conditions. The variance within groups was tripled as early feeding-rearing conditions progressed from SS to TT.

Introduction

Within the herd, several social hierarchies are known to develop which function to define priority rights to food and water and leadership for each animal. At least three (dominance, leadership and entrance order into milking parlors) and perhaps four (submission) are distinct social orders which evolve among cows. The observed social order is a function of the test situation. A submissive order which is the readiness of an animal to defer to an attacking animal is distinct from the dominance order of the provoking cow which wins. Donaldson (4) showed both the dominance and submission tendencies of dairy calves could be altered by manipulating early feeding and rearing conditions. Calves that were reared together in groups of three with limited feeding space, regardless of whether they were fed separately or with other calves, were significantly more dominant than calves reared separately. Calves that were fed together, regardless of their rearing condition were significantly more submissive than calves fed separately. Fed separate-reared separate calves were significantly less

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dominant and less submissive than other calves while fed together—raised together calves were more dominant than calves from other feeding-rearing conditions.

These same calves were available for later observations as cows to ascertain what effects early experience had upon later social and maternal behavior as well as milk producing capabilities.

Methods

The subjects in this study (5) were 18 Holstein-Friesian heifer calves and 18 Holstein-Friesian X Red Danish crossbred heifer calves. On the basis of birth order, the first nine calves born were assigned to a group (SS), the second nine were allotted to a second group, etc. until all subjects were assigned to one of four treatment conditions. The 36 calves were raised from birth until 18 weeks of age on one of the following four treatments:

- 1) Group SS: fed separately—raised separately
- 2) Group ST: fed separately—raised together
- 3) Group TS: fed together—raised separately
- 4) Group TT: fed together—raised together

An attempt was made to remove the calves from their dams within 30 minutes after birth and before they nursed. As a consequence of late night births, however, several calves were with their mothers for 4 or 5 hours and they nursed at least once. Early removal from the dam may be an important point in this study, since J. P. Scott predicted earlier that if a group of calves were taken from their mothers at birth and put together they would form primary social relationships with each other (personal correspondence with T. H. Scott, 1956). Primary social relationships in some animals are formed within a matter of hours after birth (10).

The calves were placed either individually into a 3 feet x 6 feet x 5 feet pen (0.9 m x 1.8 m x 1.5 m) or three calves lived together in a 6 feet x 12 feet x 5 feet pen (1.9 m x 2.6 m x 1.5 m). The pens provided isolation and were constructed of 3/8 inch (9.5 mm) plywood within an unheated, lighted barn. Four 6 feet x 12 feet (1.8 m x 2.6 m) feeding pens were constructed in the barn. One small 20 inch x 12 inch x 24 inch (0.50 m x 0.30 m x 0.60 m) feeder was in each feeding pen. These feeding and rearing techniques are a departure from recommended procedures at Purdue. Individual calves are normally reared and fed in a 4 feet x 7 feet pen where they can see one another. Following weaning, they are in small groups of 4-8 calves for approximately 1-2 months after which they are taken out of doors and placed in a large group of 30 heifers.

The subjects were fed a conventional powdered milk replacer, calf starter diet and a mixture of timothy and alfalfa hay. Water was available *ad libitum* in the pens. After 18 weeks of age the heifers were maintained as a group on pasture until their first parturition.

When the heifers were 20 months old agonistic or conflict behavior was observed daily for 6 weeks. A Social Index

$$\left\{ \frac{\text{number of encounters initiated}}{\text{total number of encounters}} = \text{Social Index expressed as a \%} \right\}$$

was computed for each animal. The parturition sequence was observed through two calvings during which aspects of maternal behavior were recorded. The cows were rated on milking parlor behavior by two full-time milkers. Milk production was recorded daily and 4% fat-corrected, 305 day lactation milk records were also compared for each group.

Results and Discussion

There were wide differences among cows in maternal behavior with their first calf. Raised-together cows ignored their young or partially cleaned them and failed to allow nursing. Raised-separately cows fully cleaned their offspring and allowed nursing. Raised-together cows tended not to protect their young and were quite vocal toward them while raised-separately cows were protective and did not make many vocalizations. A Chi-square test was performed for group differences in care and cleaning the calf. Values approached significance ($P < .10$) in that animals raised separately cleaned their calves better than those raised together. There were significant group differences in nursing allowed within 4 hours after birth ($P < .025$) as well as in protection of the calf ($P < .01$) and approached significance in vocalization toward the calf ($P < .10$).

With their second calf, all cows cleaned, nursed and protected their young, but there was a difference in the degree to which these behaviors were performed. Cows that had not given proper care to their first calf were not nearly as maternal as those that had taken care of their first calf. It was concluded that early feeding and rearing experiences dramatically affect maternal behavior.

It is not uncommon for cows to reject their young. Russian workers (1) support the claim that high milk-producing dairy cows and productive hens are known to lose some degree of maternal instinct. Also, Selman and co-workers (11) in Scotland, under continuous observation of natural suckling in cattle (10 beef cows, 10 dairy cows and 10 dairy heifers) during the first 8 hours postpartum, found that teat sucking advances were rejected at some time by 15 of the 30 dams. These rejections consisted of either moving away from, or kicking at, their calves. However, in 13 of the 15 dams, rejections were seen only occasionally and usually occurred only when calves pushed vigorously at udders. This mild form of rejection inhibited only the weakest calves. When suckling eventually commenced, all 13 dams became very quiet. Two primiparous heifers rejected their calves' advances for 6-8 hours. These two mothers frequently ran away from their calves.

There was no statistically significant difference among the groups in milking parlor behavior as measured by the ease of training to enter the milking parlor initially and one month postpartum, as well as

frequency of kicking, temperament and milking ease. While differences existed for other behavioral traits, one might expect milking parlor behavioral differences. However, this was not the case. The milking parlor quickly becomes a routine where defined behaviors are rewarded and all others are disciplined early and oftentimes changed. In addition, being milked and having udder pressure reduced is, in itself, rewarding and thus conducive to having a cow conform to the routine (3).

The milk production records for each breed in each feeding-rearing condition are presented in Table 1, with SS Holsteins producing up to 3,500 lbs more milk than other groups while, TT crossbreds average about 2,000 lbs more milk than other groups. With the exception of TS crossbreds, the standard deviation within groups increased as a function of increased "together" contact during early calfhood. For each breed the standard deviation is more than tripled as early feeding-rearing conditions progress from SS to TT. The TS crossbreds show the highest variance of all groups. Earlier, Donaldson (4) pointed out that the TS group (fed together—raised separately) was characterized by excessive amounts of non-nutritive sucking during their early experience, while other groups did not exhibit non-nutritive sucking after reaching 4 days of age. The only animals lost on this experiment were from group TS, one Holstein and one crossbred, who died at the age of 5 weeks.

TABLE 1. *Mean and standard deviation of milk production records of the first lactation for each breed in each feeding-rearing condition.*

Condition	Holstein- Friesian	S.D.	Crossbreds	S.D.
SS	13,381	427	10,186	546
ST	12,936	1,540	10,417	578
TS	12,068	1,307	10,453	2,614
TT	9,861	1,775	12,253	1,648
Average	12,061		10,827	

Regarding milk production, assuming a fixed model with an unweighted mean, an analysis of variance was computed. A summary of this analysis in Table 2 shows a significant difference for the breed main effect (C) with Holsteins averaging more milk produced (12,061 lbs) than crossbreds (10,827 lbs) during the first lactation. This finding was not unexpected in that purebred Holsteins have been bred and maintained for their high milk production. The crossbreds gave more milk than the smaller Red Danish, but still were not as large or capable of producing as much milk as the Holstein.

Table 2 also reveals a significant interaction of feeding by breed. An examination of the data shows that the feeding condition affected the breeds differentially. For Holsteins the fed-separate Holsteins averaged 13,158 lbs of milk while fed-together Holsteins averaged 10,964 lbs of milk. On the other hand, for crossbreds, the fed-together condi-

TABLE 2. *Analysis of variance summary for milk production.*

Source	df	MS	F	P
A (feeding)	1	2060829	.95	
B (rearing)	1	28231	.01	
C (breed)	1	9619095	4.46	.04
AB (feeding X rearing)	1	463923	.21	
AC (feeding X breed)	1	1662857	7.71	.01
BC (rearing X breed)	1	5680317	2.63	.11
ABC (feeding X rearing X breed)	1	7031519	3.26	.08
Within cell	21	2156054		

tion was more conducive to higher milk production. Fed-together crossbreds averaged 11,353 lbs of milk while fed-separate crossbreds averaged about 1,000 lbs less (10,301 lbs) of milk. By chance, higher-quality dams were used to produce the crossbred calves that filled the fed-together groups. They averaged 13,177 lbs of milk while the fed-separate calves came from dams averaging 10,800 lbs of milk.

By combining the data (ABC interaction, $P < .08$) it appears that certain conditions maximize milk production for Holsteins as well as for crossbreds.

Cows that experienced competitive raising had a higher average Social Index and were more domineering and aggressive within a herd and rejected their young at first parturition. Cows that were raised individually tended to be low ranking in the herd and accepted their young at first parturition.

The importance of "contact comfort" has been demonstrated for dogs (8, 9) and for monkeys (7). Presumably, in the absence of any type of species interaction, the SS group developed and maintained a high drive for contact which could be most fully expressed when their calves were born. If this is true, one would expect cows that have been raised individually to actively seek more contact within the herd and to maintain closer physical proximity to other animals than cows raised together. Unfortunately, such data are not available.

On the other hand, three of the groups had enriching, almost to the point of stressing, experiences in their competition for food and living space. The competitive animals experienced, in effect, an over-exaggeration of a learned behavior pattern with the ultimate effect of enhancing the frequency and efficiency of that behavior pattern.

Given that the competitive groups were inclined toward emotional disturbances due to maternal deprivation, while at the same time were prone to overreact with aggressiveness due to early experience, one can readily expect rejection of the young. Later, when other experiences had altered the effect of early experience, the second calf was accepted. In a similar hypothesis, Bronfenbrenner (2) proposes the "frustration of dependency drive" when mother-infant interactions are absent and

specifically predicts "initial fear, rejection of, and aggressiveness toward other social objects."

An alternative theory would be to ignore any deprivation during the first 4 months and focus mainly upon the agonistic experience. Cows from the competitive groups had been conditioned since the first day of their lives to be constantly perceptive of social threat. Not surprisingly they treated their first calf as another animal and only after experiencing the non-threatening aspects of the situation could they accept the second. Conversely, the raised-individually group did not perceive or respond to threat from other cows and subsequently were able to exhibit the full range of maternal behavior correctly the first time.

It would be interesting to know how early feeding and rearing experiences affect other breeds of cattle and then investigate the economic feasibility of rearing each breed in a condition that behaviorally and physiologically promotes milk production. Since twin calves are known to stay together (6), an obvious experiment is to rear twins apart from each other. They would have to be separated very early as the primary social relationships are formed within a matter of hours, *i.e.*, sheep and goats (10). It would be very interesting to see if the twins, when brought back together, would tend to stay together and imitate or mimic their behavior as they ordinarily do. The close behavioral link between twins reared together at present tends to increase the resemblance between twins in such items as grazing behavior and milk production.

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Space and Conflict in Cattle¹

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Abstract

Agonistic behavior is more pronounced among cattle when they are closely confined in yards or holding areas. Many authors have collected data on social behavior (dominance and temperament) under these conditions rather than when little agonistic behavior is occurring such as when cows are on pasture (1, 2, 3). Sommer (7) has reviewed the literature regarding dominance relationships in which a subject knows where he belongs socially, and territorially with his knowledge of a spatial place. Macmillan (5) reported that reduced fertility and short estrus cycles in young cows were the result of social stress in large herds of 200 or more cows in one group. Kilgour (4) suggested that in closely confined areas, the existence of the social order causes stress to the cows low in the dominance order in that they must constantly violate the space of higher ranking animals and they are attacked.

The purpose of the present study was to determine the amount of agonistic behavior and movement that occurs among animals in relation to rank when the size of available space is changed.

Method

Ten yearling Holstein heifers that weighed between 790 and 1,025 lbs were chosen randomly from a group of 25 maintained under summer pasturing conditions at the Purdue Dairy Center, Cherry Lane, West Lafayette, Indiana. The subjects were then confined in a 40 feet x 25 feet electric fence holding area. Adequate feed bunk space (3 feet x 16 feet) and a watering trough were at one end of the holding area.

The heifers were given 3 days to adjust to the holding area and then were observed 4 hours per day for the next 3 days to determine the social dominance hierarchy within the group, using the technique of Schein and Fohrman (6).

The holding area was lined at 5-foot intervals with a 2-inch strip of powdered lime with the result that the holding area was marked off into 5-foot squares (approximately the heifer's length of body).

One end of the fence was movable so that the size of the holding area could be adjusted by the experimenters.

Heifers were observed for 16 one-half hour periods for the next 2 days. Four observations were made under each of four sizes of the holding area: 40 feet x 25 feet, 30 feet x 25 feet, 20 feet x 25 feet

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and 10 feet x 25 feet. These areas were considered to be 100, 75, 50 and 25%, respectively, of the recommended routine pen size allocation for yearling heifers. The order of size changes were reversed with each successive observation.

During the observational periods, all agonistic behavior was recorded and the dominance order was redetermined using the technique of Schein and Fohrman (6). Also recorded was the number of squares that each subject entered. A heifer was required to put both front feet in a square to be counted as entering that space.

The dominance order was compared with the one determined previously. Data were then analyzed by means of an analysis of variance.

Results and Discussion

Assuming a fixed model, an analysis of variance was computed on the obtained scores in Table 1. A summary of this analysis in Table 2 shows significant differences for both field size and subjects ($P < .01$) in that the three low ranking animals entered the most squares, averaging 1,044, and the five middle animals entered the least squares (469). As had been predicted by Kilgour (4), low ranking animals were observed to be constantly violating the space of other animals and were moving to prevent or avoid being attacked. Surprisingly though, high ranking heifers entered an average of 215 more squares than middle ranking animals. In the smallest enclosure (10 feet x 25 feet) it appeared that high ranking animals followed the escaping low ranking subjects. Subjects in the reduced space showed less total activity ($P < .01$).

TABLE 1. *Number of squares entered by subject, space allocation and rank order.*

Subject	10 X 25 ¹	20 X 25 ²	30 X 25 ³	40 X 25 ⁴	Total	Rank Order
1	113	367	284	214	978	8
2	82	197	197	185	661	1
3	52	129	102	87	370	6
4	153	317	324	314	1,108	10
5	76	194	204	233	707	7
6	47	92	86	100	325	5
7	79	138	119	132	468	4
8	86	231	191	198	706	2
9	72	156	116	133	477	3
10	120	357	258	312	1047	9
Total	880	2,178	1,881	1,908	6,847	

¹25 ft.² per animal, *i.e.*, one 5 feet X 5 feet square per subject.

²50 ft.² per animal, *i.e.*, two 5 feet X 5 feet squares per subject.

³75 ft.² per animal, *i.e.* three 5 feet X 5 feet squares per subject.

⁴100 ft.² per animal, *i.e.*, four 5 feet X 5 feet squares per subject.

TABLE 2. *Analysis of variance of experimental data.*

	df	SS	MS
Subjects	9	44781.26	4975.69
Fields	3	24408.92	8136.30
S X F	27	8897.27	329.52
Replicates	3	8845.02	2948.34
Error	117	58929.73	503.67
Total	159	145862.20	

F (Subjects) = 9.87**

F (S X F) = Non Significant

F (Fields) = 24.69**

** P < .01

Movement can be categorized as another indication of dominance. The behavior and status of animals moving from one area to another area was remarkably similar.

Considerable movement, conflict, competition and agonistic behavior occurred near the feed bunk. This is similar to earlier work with heifers (1, 3) and with cows (2).

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A Check List of Freshwater Ostracods of Indiana— Including Sixteen New Recordings and a Description of Two New Species

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Abstract

The check list of freshwater ostracods of Indiana raises the number of ostracods reported from Indiana from 6 to 24. The list is a result of a survey taken in the spring of 1968 in Delaware County. The list includes the descriptions of 2 new species: *Potamocypris brachychaeta* and *Candona ginnensis*.

Introduction

A survey of ostracods (Class Crustacea, Order Ostracoda) was taken through Delaware County during May and June, 1968. The survey was an attempt to fill a gap in the taxonomy of ostracods from Indiana. Comprehensive surveys of ostracod faunas have been made in Ohio (1) and Illinois (3), but to date none have been made in Indiana. There are scattered reports of ostracods from Indiana; among these are 4 cave types; *Candona marengoensis*, *C. jeanneli* (5), *Entocythere donaldsonensis* (7), *E. barri* (2). There are only 2 ostracod species reported from streams and ponds in Indiana: *Cypridopsis vidua* (4), *Cyclocypris laevis* (6). The survey of Delaware County raises the number of known ostracods from Indiana from 6 to 24 including 2 new species.

Methods and Materials

During May and June 1968, 100 dredge-net samples were taken in Delaware County. The samples were taken from rivers, creeks, ponds, drainage ditches, and borrow pits. Of the 100 samples 62 contained ostracods. The ostracods were preserved in a 95% alcohol solution. Permanent reference slides were made and placed on file in the Ball State University biology laboratory.

Description of New Species

Potamocypris brachychaeta, sp. nov.

Five specimens were collected from two sample sites.

Sample number 20 was from a borrow pit along Interstate 69 in Delaware County. The pit is about 6 acres in area and the true depth is not known. The bottom is mostly mud and silt. There was little vegetation except along the edges of the pit, and this was mostly grass and unidentified algae. *Cypridopsis vidua* was also found in the sample.

Sample 31 was taken from a small creek with slow movement under normal conditions. The bottom was mostly silt and much decaying vegetation. The water was clear. Five other species of ostracods were found at this site: *Ilyocypris bradyi*, *Candona elliptica*, *Candona* sp., *Paracandona* sp., and *Darwinula stevensoni*. Also found in the collection were many unidentified insect larvae, copepods, and cladocera.

Potamocypris brachychaeta is easily identified as genus *Potamocypris* because of the genus characteristics: reniform shape, compressed valves, arched dorsum maximum height

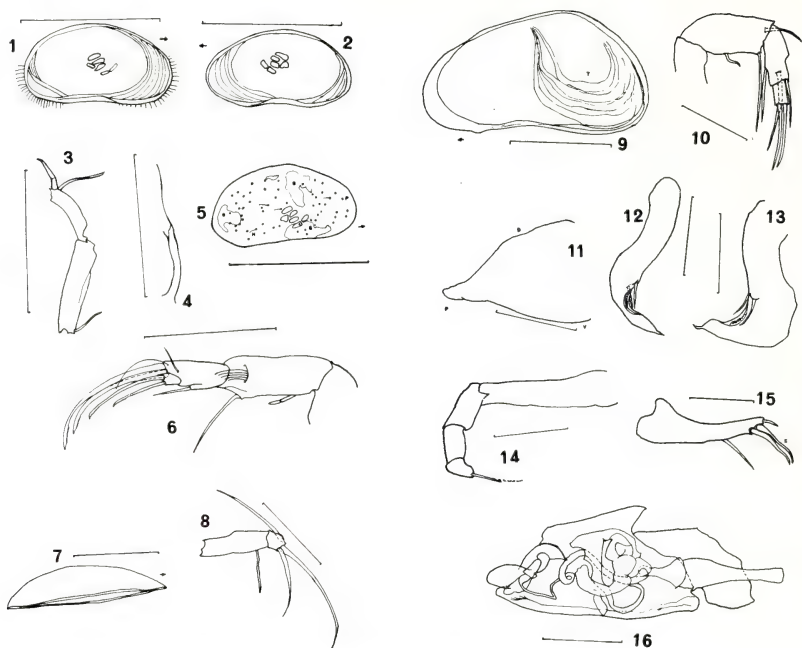


FIGURE 1. *Potamocypris brachychaeta*. Interior view of left valve showing list. Scale 0.5 mm. Arrow—anterior.

FIGURE 2. *P. brachychaeta*. Interior view of right valve. Scale 0.5 mm.

FIGURE 3. *P. brachychaeta*. Third leg. Scale 0.1 mm.

FIGURE 4. *P. brachychaeta*. Furca. Scale 0.1 mm.

FIGURE 5. *P. brachychaeta*. Exterior view of right valve. Scale 0.5 mm. G—green, H—hair, P—pit.

FIGURE 6. *P. brachychaeta*. Second antenna. Scale 0.1 mm.

FIGURE 7. *Candona ginnensis*. Dorsal view of left valve. Scale 0.5 mm.

FIGURE 8. *C. ginnensis*. Third leg. Scale of 0.1 mm.

FIGURE 9. *C. ginnensis*. Interior view of right valve. Scale 0.5 mm. T—testes.

FIGURE 10. *C. ginnensis*. Second antenna. Scale 0.1 mm.

FIGURE 11. *C. ginnensis*. Genital lobe. Scale 0.1 mm. V—ventral, P—posterior, D—dorsal.

FIGURE 12. *C. ginnensis*. Oblique view of left prehensile palp showing the teeth. Scale 0.1 mm.

FIGURE 13. *C. ginnensis*. Right palp. Scale 0.1 mm.

FIGURE 14. *C. ginnensis*. Leg 2. Scale 0.1 mm. Dashed line shows normal length of terminal seta.

FIGURE 15. *C. ginnensis*. Furca. Scale 0.1 mm. S—S-shaped seta.

FIGURE 16. *C. ginnensis*. Penis. Scale 0.1 mm.

reached at mid-length, broad anterior vestibule, with the left valve slightly overlapping the right valve. The valves of *P. brachychaeta* are punctate with very few hairs and are grayish with several areas of green pigment. The pore canals are simple and the hinge is adont. This new species differs from the other potamocyprids with abbreviated natatory setae in that the setae of *P. brachychaeta* are very short (Fig. 6). The swimming setae are a mere 0.08 mm long and barely reach the proximal 1/3 of the penultimate segment. Another distinguishing feature is the appearance of a second posterior list on the left valve (Fig. 1). This list does not appear on any of the other species of *Potamocypris*.

Dimension of adult female: Right valve 0.56 mm long, 0.29 mm high. Left valve 0.59 mm long, 0.36 mm high. Width 0.3 mm.

Candona ginnensis, sp. nov.

The sample was collected from Ginn's Woods, a preserve of Ball State University in Delaware County, Indiana. Five individuals were found in a sample taken in April. The sample contained *Candona truncata* and the eggs of the spring peeper, *Hyla crucifer*, and the chorus frog, *Pseudacris nigrita*.

The left valve of *Candona ginnensis* is slightly longer and higher than the right valve. The greatest height is reached in the posterior one-third of the valve. From the rounded anterior, the dorsal margin arches gradually to the maximum height. Posteriorly the dorsal arch curves gently to the posterior; this downward curve is not sharp as in *Candona truncata*. From the broadly rounded posterior margin the ventral margin forms a slight sinuation at the mid-point. The male has a weak point where the ventral margin meets the anterior margin. The third leg has an undivided penultimate segment. The prehensile palps are both similar with a double row of teeth on the ventral side at the curve between the setae (Figs. 12, 13). The furca has a definite "S" shape in the female (Fig. 15) and is only slightly less pronounced in the male.

Dimensions of adult female: Right valve 0.85 mm long, 0.42 mm high. Left valve 0.93 mm long, 0.45 mm high. Width about 0.5 mm.

Dimensions of adult male: Right valve 1.1 mm long, 0.5 mm high. Left valve 1.2 mm long, 0.6 mm high. Width not determined.

Candona ginnensis is similar to *Candona simpsoni* (Sharp, 1897) and *C. sigmoides* (Sharp, 1897) (6) with each having an S-shaped furca. However, the valves of *Candona ginnensis* and *C. sigmoides* are arching, while the valves of *C. simpsoni* are elliptical in shape. The sizes also differ with *Candona ginnensis* being between *C. simpsoni*, the smallest, and *C. sigmoides*, the largest. The penis of *C. ginnensis* (Fig. 16) is longer and somewhat narrower than the penis of *C. sigmoides*. The genital lobe (Fig. 11) of the female of the new species is shorter and less pointed than that of *C. sigmoides*.

A Check List of Freshwater Ostracods of Indiana

Class Crustacea

Order Ostracoda

Suborder Podocopa

Family Darwinulidae

Darwinula stevensoni (Brady and Robertson, 1870)

Family Cypridae

Subfamily Ilyocyprinae

Ilyocypris bradyi (Sars, 1890)

I. gibba (Ramdohr, 1808)

Subfamily Cyprinae

Cyprinotus sp. (Brady, 1886)

Subfamily Cypridopsianae

Cypridopsis vidua (O. F. Müller, 1776)

Potamocypris smaragdina (Vavra, 1891)

P. brachychaeta, sp. nov.

Subfamily Candocyprinae

Tribe Cyclocyprini

Physocypria globula (Furtos, 1933)*Cypria maculata* (Hoff, 1942)*C. obesa* (Sharp, 1897)*C. turneri* (Hoff, 1942)

Tribe Candonini

Candona elliptica (Furtos, 1933)*C. stagnales* (Sars, 1890)*C. marengoensis* (Klie, 1931)*C. jeanneli* (Klie, 1931)*C. ginnensis*, sp. nov.

Subfamily Cyprinae

Genus *Cypris*Subgenus *Eucypris* (Vávra, 1891)*E. affinis hirsuta* (Fischer, 1851)*E. reticulata* (Zaddach, 1844)

Subfamily Candocyprinae

Cyclocypris laevis (Müller, 1912; Sharp, 1918)*C. forbesi* (Sharp, 1897)

Family Cytheridae

Subfamily Entocytherinae

Genus *Entocythere* (Marshall, 1903)*E. donaldsonensis* (Klie, 1931)*E. barri* (Hart and Hobbs, 1961)*E. sp.*

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Parasites of the Bullfrog in Indiana¹

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Abstract

Parasitological examinations were made of 72 bullfrogs from four Indiana areas during May through September 1970, of which 91.7 per cent contained parasites. Parasites found included: (protozoans) *Hexamita intestinalis*, *Nyctotherus cordiformis*, *Trypanosoma rotatorum*, and an unidentified species of *Trichomonas*; (trematodes) *Gorgoderia amplicava*, *Gorgoderina attenuata*, *Haematoloechus breviplexus*, *Haematoloechus longiplexus*, and *Glypthelmins quita*; and (nematodes) *Cxysomatium longicauda* and one unidentified species. Two species of Hirundinae, *Placobdella rugosa* and *Glossiphonia stagnalis*, were found apparently as parasites.

Introduction

Brandt (2), in a comprehensive study of the parasites of several species of North Carolina frogs, has presented the only extensive review of the parasites of bullfrogs, *Rana catesbeiana* Shaw. He found the bullfrog to harbor a number of helminth and protozoan parasites and one arthropod. Reports of parasites of bullfrogs in Indiana are few. Cort (5) observed the lung flukes *Haematoloechus longiplexus* and *H. breviplexus* from a North Judson, Indiana, bullfrog, and Walton (21) found cysts of the nematode *Physaloptera ranae* in the stomach and intestinal walls in bullfrogs of northern Indiana. Krull (11) also found *H. longiplexus* in an Indiana bullfrog. More recently, Myer (17) reported metacercariae of the fluke *Mesostephanus kentuckiensis* in Ohio bullfrogs. Since Cable (3) reported cercariae of *M. kentuckiensis* in snails from McCormick's Creek and Clifty Creek, Indiana, this trematode may occur in Indiana bullfrogs, although none has yet been reported.

Materials and Methods

The bullfrogs examined in this study were collected from four Indiana Fish and Game Areas: Glendale, in southwestern Indiana (Daviess County), Crosley Lake in the southeast (Jennings County), Willow Slough in the northwest (Newton County), and Tri-County in the northeast (Kosciusko and Noble Counties). Frogs were collected at night by hand or by a dip net.

Five monthly collections were made at each of the four study areas. The frogs were taken to the laboratory at Ball State University and placed in a refrigerator until examined. Some frogs were dissected and searched for parasites within 24 hours after collection, but others were held for as long as 10 days before examination.

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Before examination the bullfrogs were either pithed or beheaded. The heart, lungs, stomach, large and small intestines, cloaca, liver, gall bladder, kidneys, and urinary bladder were examined. Before removing the organs, the host was searched for any coelomic parasites, using a dissecting microscope. Upon removal, each organ was placed individually in frog Ringer's Solution in a watch glass in order to retain any parasites which might leave the organ.

Each organ was then opened or teased apart under a binocular dissecting microscope. Except for protozoans, all parasites found were counted and representatives preserved for permanent mounting. Smears were made of the contents of the intestines and of peripheral, renal, and cardiac blood for examination under a compound microscope.

Blood smears were made by using Wright's stain or by fixing in 95% alcohol and staining with Giemsa's stain. Intestinal smears were fixed in Schaudinn's fluid and stained with Heidenhein's iron haematoxylin.

All worms were washed thoroughly in physiological saline before fixing. Trematodes were relaxed in distilled water and then fixed in either 10% formalin, formol-acetic-alcohol (F.A.A.), or Bouin's solution which had been heated to bubbling but not vigorous boiling. Nematodes were dropped into warm F.A.A., 10% formalin, or 70% acid-alcohol for fixation. Leeches were fixed in 70% alcohol using the method described by Mann (14). For permanent storage, all worms were transferred to either 70% alcohol plus 5% glycerine or 5% formalin plus enough glycerin to make a 5% solution, depending upon the method of fixation used.

Stains used for trematodes and nematodes included Grenacher's Alcoholic Borax-Carmine, Mayer's MCl Carmine, either with or without Fast Green counterstaining, and Harris' or Delafield's Haematoxylin. After staining, the specimens were destained, dehydrated, cleared, and mounted in Permount.

Cort (4, 5), Harwood (8), Higgins (9), Kudo (12, 13), Mann (14), Miller (16), Miller (15), Najarian (18), and Yamaguti (23) were used for making identifications.

Results

Seventy-two bullfrogs were examined for parasites between May and September of 1970, 20 each from Glendale and Crosley Lake and 16 each from Willow Slough and Tri-County. Those frogs 100 mm or more in snout-vent length were classed as adult, and those below 100 mm were considered juveniles. According to Wright and Wright (22), 100 mm is approximately the minimum breeding size. The range of length of the 49 adult frogs examined was 100 mm to 142 mm, with an average length of 115.6 mm. The 23 juveniles ranged from 47 mm to 77 mm, the average being 62 mm.

Of the 72 bullfrogs examined, 91.7% were found to be infested by one or more species of parasites (Table 1). Four species of protozoans

TABLE 1. *Per cent of bullfrogs infested, by size, collection areas, and month of collection.*

Number of frogs examined	Size			Collection Areas				Month of Collection				
	Adult (49)	Juv. (23)	Total (72)	C.L. (20)	Glen. (20)	T.C. (16)	W.S. (16)	May (9)	June (19)	July (17)	August (13)	Sept. (14)
<i>Hexamita intestinalis</i>	69.4	87.0	75.0	85.0	95.0	62.5	50.0	55.6	68.4	82.4	76.9	85.7
<i>Trichomonas</i> sp.	85.7	87.0	86.1	85.0	90.0	68.75	93.75	66.7	84.2	100.0	92.3	78.6
<i>Nyctotherus cordiformis</i>	34.7	30.4	33.3	45.0	20.0	25.0	43.75	44.4	42.1	29.4	30.8	21.4
<i>Trypanosoma rotatorum</i>	6.12	4.35	5.56	0.0	0.0	18.75	6.25	0.0	5.26	17.65	0.0	0.0
<i>Gorgoderu amplicava</i>	28.6	13.0	23.6	5.0	30.0	18.75	43.75	11.1	10.53	41.2	30.8	21.4
<i>Gorgoderina attenuata</i>	53.1	13.0	40.3	45.0	55.0	43.75	12.5	33.3	47.4	29.4	46.2	28.6
<i>Haematoloechus breviplexus</i>	22.5	13.0	19.4	10.0	0.0	37.5	37.5	0.0	15.8	23.5	15.4	21.4
<i>Haematoloechus longiplexus</i>	14.3	17.4	15.3	5.0	50.0	0.0	0.0	0.0	10.53	23.5	0.0	7.14
<i>Glyphelminis quita</i>	8.16	8.7	8.33	0.0	10.0	18.75	6.25	11.1	15.8	11.8	0.0	0.0
<i>Oryzomatium longicaudata</i>	10.2	4.35	8.33	10.0	15.0	6.25	0.0	0.0	10.53	17.65	7.69	0.0
Nematode (unidentified)	2.04	0.0	1.39	0.0	0.0	0.0	6.25	0.0	0.0	5.88	0.0	0.0
Leeches ²	0	0	0	0	0	4	24	6	11	10	1	0

²Actual number of leeches found.

were found: *Hexamita intestinalis*, *Nyctotherus cordiformis*, *Trypanosoma rotatorum*, and an unidentified *Trichomonas* sp. Trematodes included the bladder flukes *Gorgodera amplicava* (3.817)² and *Gorgoderina attenuata* (0.772), the lung flukes *Haematoloechus breviplexus* (0.780) and *H. longiplexus* (0.793), and the intestinal fluke *Glypthelmins quieta* (1.913). The nematodes found were *Oxysomatium longicaudata* (2.822) and one unidentified species. No cestodes or acanthocephalans were encountered.

Two species of leeches, *Placobdella rugosa* and *Glossiphonia stagnalis*, were found in the collecting jars, but were never seen attached to the frogs. Probably the leeches were on the frogs at the time of collection.

Discussion

In addition to the primary species-specific limitations, many factors influence the kinds and numbers of parasites in a host: age, geographic distribution, diet, the presence of suitable intermediate hosts, seasonal aspects of the life cycle, etc. The operation of some of these factors as they apply to parasitism in the bullfrog has been reported by other authors, and data from the present study suggest the operation of others. For example, bullfrogs were introduced into the Rocky Mountain region relatively recently, but their parasites do not yet seem to be well established there (20). This may well be due to the absence of suitable intermediate hosts.

Although the samples are small, the data in Table 1 suggest some seasonal variation in the abundance of parasites, most species showing peaks of occurrence in June, July, or August. Leeches were most abundant in June and July, and the recording of trypanosomes only in these 2 months may well be related to the abundance of the leeches, their possible intermediate hosts.

Although most parasites were found in all four collecting areas (Table 1), there were two possibly significant exceptions. The blood protozoan, *Trypanosoma rotatorum*, was found in only the two northern collecting areas. This may again be due to the presence in these areas of large numbers of leeches. Leeches have been reported as intermediate hosts of trypanosomes in certain other amphibians: *Trypanosoma diemyctyli* in the Red-spotted Newt, *Notophthalmus viridescens* (1), and in various European frogs (6).

The other exception is the lung fluke *Haematoloechus longiplexus*, which was found only in the two southern areas.

With respect to adult frogs *versus* juveniles, only in two species of parasites is there a notable difference in the overall amount of infestation. *Hexamita intestinalis* was found more often in juvenile bullfrogs than in adults (86.9% incidence compared to 69.5%). The

²Average number of parasites per individual bullfrog.

bladder fluke, *Gorgoderina attenuata*, was found in over half (53.1%) of the adult frogs, but in very few of the smaller frogs (13%).

Multiple infestation in bullfrogs is a common occurrence and has been reported by Cort (5), Miller (15), and Najarian (18). In this study, some individual frogs contained six different species of parasites: three of the four protozoans and three of the five trematodes, although a typical frog would harbor one or two species of protozoans and a species of fluke.

Two species of leeches, *Placobdella rugosa* and *Glossiphonia stagnalis*, are here reported for the first time as parasites of bullfrogs.

Acknowledgments

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Distribution of Free-living Soil Nematodes of the Belondiroidea in Indiana and the United States¹

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Abstract

During a 5-year study of distribution of soil and fresh-water nematode species in Indiana, four genera of the Belondiroidea have been found widely distributed in natural areas of the state. Of these, *Dorylaimellus* Cobb, *Axonchium* Cobb, and *Oxydirus* Thorne were found in most biotic units of the state. In subsequent sampling in widely separated areas of the U. S., *Dorylaimellus* and *Axonchium* were found in natural areas in all sections of the country sampled. Species of *Oxydirus* were found less frequently and appeared in only one collection in western U. S., a forest meadow in Sequoia National Park, California. Although four species of *Belondira* Thorne were well-distributed in southern Indiana, only two were found as far north as Tippecanoe County. Despite extensive sampling throughout the U. S. we have recovered specimens of this genus from a very limited geographic area, extending only from eastern Kansas to Tennessee.

Introduction

The nematode superfamily Belondiroidea as presently understood by most authorities consists of eight families and 13 genera (3). Of these, only five genera, one in each of five families, have been reported from continental United States: *Dorylaimellus* Cobb in the Dorylaimellidae; *Axonchium* Cobb in the Axonchiidae; *Oxydirus* Thorne in the Oxydiridae; *Belondira* Thorne in the Belondiridae; and *Swangeria* Thorne in the Swangeriidae. All contain stylet-bearing soil species whose food habits are largely unknown. Some may be plant parasitic, although they are not generally found in large numbers around cultivated plants at least in temperate regions. For this reason they have been studied very little.

Dorylaimellus virginianus Cobb was originally found by Cobb (2) in Virginia and subsequently also in Utah by Thorne (8). Other species of *Dorylaimellus* were found by Thorne (8) and Cobb (2) in Virginia, Idaho, Utah, Texas, and Colorado; and by Orr and Dickerson (7) in Kansas. A wide distribution for species of *Axonchium* was recorded by Thorne (8) when he reported their occurrence in Utah, Colorado, Missouri, Washington, D. C., Georgia, South Carolina, and Florida. More recent collections were noted by Orr and Dickerson (7) in Kansas and by Hechler (5) in Virginia. *Oxydirus* spp. were reported from Utah and Virginia by Thorne (8), and Hechler (4) reported finding *Oxydirus gangeticus* Siddiqi in Maryland. A single species of *Belondira* (*B. apitica* Thorne) was found by Thorne (8) in Utah, and this species plus another were found by Orr

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and Dickerson (7) in Kansas. *Swangeria bisexualis* Hopper was described (6) from a series of 10 specimens taken in Florida.

Despite the fact that the occurrence of belondiroid nematodes in the U. S. has been infrequently noted by nematologists, we found four of the five genera discussed above to be common in Indiana. Subsequent sampling over the rest of the country indicated that these four genera differ from each other in their biogeography. The present report concerns the distribution of genera only. Detailed studies of the species found within each genus will follow in later papers.

Methods

Intensive sampling of natural sites within the biotic units of Indiana (1) was carried out over a 5-year period. Sites were usually wooded and often included a stream or river. The number of samples taken at any given time from a specified area varied from 6 to 50, but most areas were sampled several times at different seasons of the year. Three 3-liter samples were collected from each site. One sample was collected from the edge of the stream or river using a 2.5 cm sampling tube thrust 15-20 cm into the soil, whenever possible. Approximately 50 such cores were collected for a distance of 30 m along the contour of the stream. A second such sample was taken from the normal flood plain, but away from the stream edge; and a third sample was taken well outside the normal flood plain. Additional samples were taken in specialized and typical habitats in all areas. Detailed ecological data were recorded at the time of sampling including information about flora of the area, soil type, soil contour, soil moisture, and other factors. Samples from other parts of the United States were collected in a manner similar to that used for Indiana samples.

In the laboratory one 1-liter subsample was processed from each sample by a combination of sieves and Baermann funnels. Nematodes were relaxed by gentle heat (57°C for 10 min.) and killed and fixed in F. A. A. (9). A "profile" of all species present was established for each sample and the specimens processed to permanent glycerine mounts by the Thorne slow method (9).

Results and Discussion

The occurrence of representatives of these four genera in our collections is shown on the maps, Figure 1 (Indiana) and Figure 2 (the U. S.). *Dorylaimellus* species are common throughout Indiana and are present in all areas of the U. S. we have sampled. The same generalization may be made about *Axonchium*, although it should be noted that we have recovered fewer species of *Axonchium* than of *Dorylaimellus* (3). Nematodes belonging to the genus *Oxydirus* are found in all parts of Indiana, although they were less numerous than nematodes of the former two genera. *Oxydirus* specimens appeared in only one collection in western U. S., a forest meadow in Sequoia National Park, California.

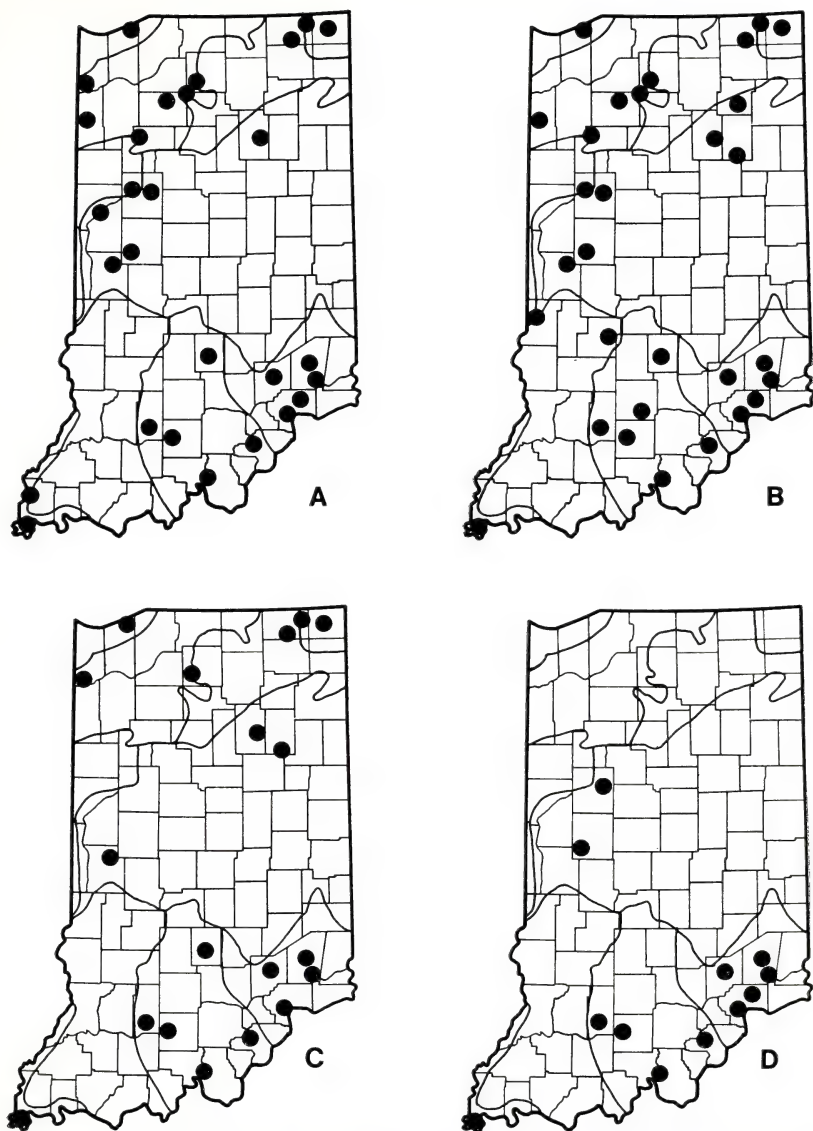


FIGURE 1. Distribution of four genera of the Belondiroidea in Indiana (map is divided into biotic units of Chandler (1)). A, *Dorylaimellus*; B, *Axonchium*; C, *Oxydirus*; D, *Belondira*.

Although four species of *Belondira* are well-distributed in the southern part of Indiana, only two species were found as far north as Tippecanoe County. Despite a vigorous search for representatives of this genus throughout the U. S., we have recovered specimens from a very limited geographic area, extending only from eastern Kansas to Tennessee.

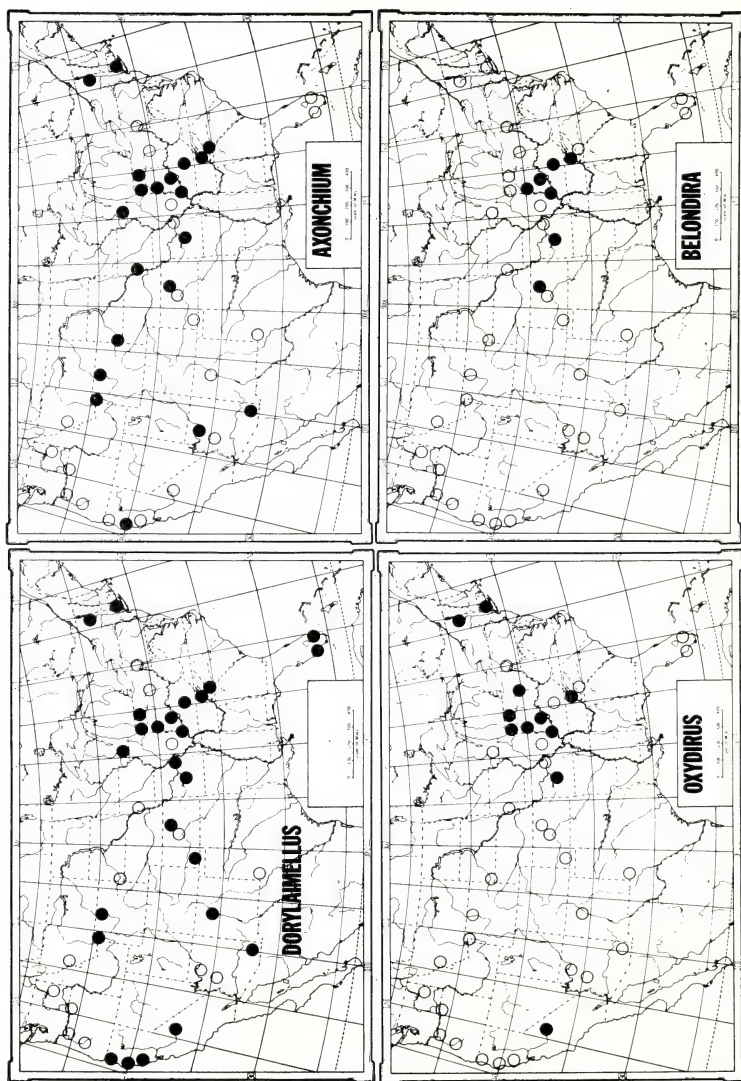


FIGURE 2. Distribution of four genera of the Belondiroidea in the United States. Open circles indicate sites sampled, and solid circles show where a given genus was found.

Conclusions

Although members of the Belondiroidea have never been reported from many areas of the new world, it appears that the genera *Dorylaimellus* and *Axonchium* are widely distributed and common throughout the U. S. *Oxydirus* appears to be less widely distributed, especially in the western states, and the genus *Belondira* appears to be restricted in distribution.

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The Woodrat in Indiana: Recent Fossils

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Abstract

Skeletal remains of *Neotoma*, presumably *N. floridana magister*, have been recovered in relative abundance from caves throughout Indiana's karst area associated with bones of such locally extinct fauna as black bear, porcupine, elk, and spotted skunk. Presently rare in Indiana, a few woodrats have been historically recorded and recently observed marginal to the Ohio River. The ancient depopulation may have been associated with some indirect effect of climatic change.

One species and several races of woodrat are present in the Eastern United States (4). Only a few woodrats, *Neotoma floridana magister*, have been recorded in Indiana (7), along and near the bluffs of the Ohio



FIGURE 1. Locations of *Neotoma* observations (circles) and "recent fossil" sites (squares).
[1] Freeman's Pit, Hidden Pit Cave; [2] Beetree Cave, Brinegar's Cave; [3] Anderson Pit Cave; [4] Sullivan's Cave (1); [5] Carcass Crypt Cave; [6] Stroud Cave (6); [7] N. Jim Cave; [8] Cave 3 miles S.S.W. of North Vernon; [9] Wyandotte Cave (2, 8); [10] Small bluff-top cave (5); [11] Rat Cave (5).

River (5), and in Wyandotte cave (2, 8). The author has recently noted woodrats in the two caves near the Ohio River where Hickie and Harrison first recorded them in 1930 (5).

Kirkpatrick and Conaway found woodrat nests, though no rats, in Stroud cave, Orange County (6). Bader and Hall (1) found remains of woodrat in Sullivan's cave, Lawrence County, associated with bones of *Didelphis marsupialis*, *Blarina brevicauda*, *Erethizon dorsatum*, *Sylvilagus floridanus*, *Spilogale putorius*, *Procyon lotor*, *Urocyon cinereoargenteus*, *Odocoileus virginianus*, *Cervus canadensis*, and *Myotis* sp., again indicating an ancient distribution farther north than at present.

TABLE 1. Location and occurrence of *Neotoma* remains.

Cave	Quadrangle, County	Occurrence and Important Associations
Freeman's Pit	Whitehall, Monroe	A dozen <i>Neotoma</i> with <i>Ursus</i> , <i>Spilogale</i> , and <i>Erethizon</i> in the explored top 17 inches of an osteiferous, laminated silt-clay deposit.
Hidden Pit Cave	Whitehall, Owen	One <i>Neotoma</i> in the top 4 inches of the silt floor of a dome.
Brinegar's Cave	Stanford, Monroe	Two <i>Neotoma</i> , one in a small nest with 2 <i>Ursus</i> .
Beetree Cave	Stanford, Monroe	One <i>Neotoma</i> in a nest, and another in a silt with <i>Erethizon</i> near an old entrance.
Trap Door Cave	Stanford, Monroe	A large nest with few bones.
Anderson Pit Cave	Clear Creek, Monroe	A large nest with 8 <i>Neotoma</i> and a human parietal fragment. Another nest was largely of vegetal material.
Sullivan's Cave	Owensburg, Lawrence	A large nest with 7 <i>Neotoma</i> , <i>Ursus</i> , <i>Erethizon</i> , and <i>Cervus</i> . Possibly the nest of Bader and Hall (1). Several other nests in the cave.
Carcass Crypt Cave	Bedford West, Lawrence	A nest with large <i>Ursus</i> bones and one <i>Neotoma</i> . Five <i>Neotoma</i> were contemporaneously associated with <i>Ursus</i> , <i>Spilogale</i> , and <i>Didelphis</i> in a carbon-rich area overlain by a sterile silt-clay layer.
Stroud Cave	Valeene, Orange	One <i>Neotoma</i> with <i>Erethizon</i> , among several nests.
N. Jim Cave	Mauckport, Harrison	One <i>Neotoma</i> in an osteiferous, fissure in-washed silt deposit. A <i>Spilogale</i> dentary nearby was not necessarily associated.
Cave near North Vernon	Hayden, Jennings	<i>Neotoma</i> skull and <i>Ursus</i> tooth on surface near the entrance.

From Indiana's caves have recently come numerous "Recent fossils" of *Neotoma* (Table 1), some as far north as western Monroe county (Fig. 1), and many with extensive associated faunas (Tables 2 and 3).

Discussion

Neotoma distribution in the past was most likely concurrent with the limits of both of Indiana's karst areas, according with its cave and

TABLE 2. *Fauna recovered from the various Neotoma nests.*

Species	Beetree Cave ¹	Anderson Pit Cave ²	Sullivan's Cave ³	Stroud Cave ⁴
<i>Myotis sp.</i>	27	61	95	3
<i>Pipistrellus subflavus</i>	3	3	23	1
<i>Peromyscus sp.</i>	8	47	28	5
<i>Microtus sp.</i>	3	25	12	6
<i>Neotoma floridana</i>	1	8	7	1
<i>Blarina brevicauda</i>	4	30	28	4
<i>Sciurus sp.</i>	2	3	2	1
Indet. mole	1	3	1	1
<i>Bufo sp.</i>	1	4	1	—
Indet. frogs	2	2	2	—
Plethodontidae	2	2	5	4
Colubridae	1	3	2	1
Indet. bird	—	3	2	1

¹Also from Beetree Cave were: Indet. large bat, 1; Indet. bat, 13; *Synaptomys cooperi*, 2; *Tamias striatus*, 3; *Marmota monax*, 1; Indet. small carnivore, 1; *Sylvilagus floridanus*, 1; *Odocoileus virginianus*, 1; *Ambystoma sp.*, 1; Crotalidae, 2; Indet. fish, 1; and snails.

²Also from Anderson Pit Cave were: Indet. large bat, 1; *Synaptomys cooperi*, 3; *Tamias striatus*, 1; *Marmota monax*, 1; *Sorex sp.*, 3; *Procyon lotor*, 2; Indet. small carnivore, 1; *Didelphis marsupialis*, 1; *Sylvilagus floridanus*, 1; *Odocoileus virginianus*, 2; *Homo sapiens*, 1; *Ambystoma sp.*, 1; *Terrepene sp.*, 1; Crotalidae, 1; Indet. fish, 1-3 (?); snails; crayfish; and small clams.

³Also from Sullivan's Cave were: *Eptesicus fuscus*, 1; Indet. bats, 6; *Synaptomys cooperi*, 1(?); *Tamias striatus*, 1; *Marmota monax*, 1; *Erethizon dorsatum*, 1; *Ursus americanus*, 1; *Procyon lotor*, 2; *Urocyon cinereoargenteus*, 1; Indet. small carnivore, 1; *Didelphis marsupialis*, 1; *Sylvilagus floridanus*, 1; *Odocoileus virginianus*, 1(?); *Cervus canadensis*, 1; *Ambystoma sp.*, 2; Crotalidae, 2; Indet. fish, 3-6 (?); aquatic snails; crayfish; small clams; bird eggshell (?).

⁴Also from Stroud Cave were: Indet. large bat, 1; *Marmota monax*, 2; *Erethizon dorsatum*, 1; *Procyon lotor*, 1; *Didelphis marsupialis*, 1; *Sylvilagus floridanus*, 1; *Odocoileus virginianus*, 1(?); Indet. frog or toad, 3; *Terrepene sp.*, 1; Naticrinae, 1; Indet. fish, 1; snails; crayfish; small clams.

bluff crevice habitat. Nests were observed in the wall solution anastomoses, in fissures, crevices, on ledges, and in breakdown piles; *Neotoma* bones, however, occurred in many depositional situations. Nests seem to have been used over again by successive woodrats. With time, the vegetal materials of the nest disintegrate, concentrating the bones, rocks, snails, etc., emphasizing the faunal component, whereas in all actively inhabited nests observed by the author in Kentucky the floral component had hundreds of times more bulk. Thus, the older nests usually had the greater bone concentration. The floral contents of nests have yet to be studied. Of the faunal contents, only those bones actually chewed upon can be attributed to the woodrat's collecting behavior, these being most all of the larger than squirrel-sized forms, with deer bones often making up the bulk. The extensive microfaunal remains were predominantly those animals inhabiting the cave (e.g.,

TABLE 3. Faunal remains from the various cave deposits.

Species	Hidden Pit Cave ¹	Brinegar's Cave ²	Beetree Cave ³	Carcass Crypt Cave ¹	N. Jim Cave ⁵	Cave, N. Vernon ⁶
<i>Myotis</i> sp.	2	—	28	395	360	—
<i>Pipistrellus subflavus</i>	5	—	2	6	52	—
<i>Peromyscus</i> sp.	26	—	5	11	192	—
<i>Microtus</i> sp.	—	—	5	4	1	—
<i>Neotoma floridana</i>	1	2	1	5	1	1
<i>Tamias striatus</i>	3	—	3	4	4	1(?)
<i>Blarina brevicauda</i>	2	1	6	3	5	—
<i>Sorex</i> sp.	1	—	—	1	23	—
<i>Bufo</i> sp.	1	—	5	2	—	2
Plethodontidae	4	—	2	2	22	—
Colubridae	2	—	2	2	ca. 17	—
Indet. bird	—	1	2	2	1	3

¹Also from Hidden Pit Cave were: Indet. bats, 2; *Sciurus* sp., 1; *Marmota monax*, 1; *Ambystoma* sp., 1; snails.

²Also from Brinegar's Cave were: *Sciurus* sp., 1; *Ondatra zibethicus*, 1; Indet. mole, 1; *Ursus americanus*, 2; *Mephitis mephitis*, 1; *Odocoileus virginianus*, 1; *Terrepene* sp., 1.

³Also from Beetree Cave were: Indet. bats, 9; *Sciurus* sp., 1(?); *Erethizon dorsatum*, 1; *Scalopus aquaticus*, 1; Indet. canidae, 1; Indet. small carnivore, 1; *Sylvilagus floridanus*, 1; *Odocoileus virginianus*, 1; *Ambystoma* sp., 1; Crotalidae, 2; Indet. lizard, 1; Indet. fish, 1; snails.

⁴Also from Carcass Crypt Cave were: Indet. large bat, 1; Indet. bats, 7; *Sciurus* sp., 2; *Erethizon dorsatum*, 1(?); Indet. shrew, 3; Indet. mole, 1; *Ursus americanus*, 2; *Procyon lotor*, 1; *Spilogale putorius*, 1-2; *Mephitis mephitis*, 1; *Didelphis marsupialis*, 1; Indet. frog, 4; *Ambystoma* sp., 1; *Terrepene* sp., 1; Crotalidae, 1; snails.

⁵Also from N. Jim Cave were: *Eptesicus fuscus*, 5; Indet. bats, 24; Indet. frogs, 2; *Ambystoma* sp., 1; Crotalidae, 2; Indet. lizard, 1; snails.

⁶Also from the Cave near North Vernon were: *Lasiurus* sp(?) 1; *Sciurus* sp., 1; *Marmota monax*, 1; Indet. mole, 1; *Ursus americanus*, 1; *Procyon lotor*, 2; *Didelphis marsupialis*, 1; *Sylvilagus floridanus*, 3; *Odocoileus virginianus*, 2; Indet. frog, 1; Indet. turtle, 2; Indet. snake, 1; Indet. fish, 1; large clams.

mice, shrews, bats, salamanders) or trapped in it (e.g., toads, frogs, snakes) and attracted to the nest, as indicated by the completeness of their skeletons, lured by the numerous invertebrates infesting its organic contents. Dying bats probably fell into the nests from their roosts. The minute clams and crayfish remains seem to be unique to the nests, perhaps collected by the woodrats, though along with the fish, could also been in the stomachs of raccoons, snakes, etc. Thus, many of the forms from the nests probably post-date the *Neotoma*, being only pene-contemporaneous. Animals associated in the sedimentary deposits, however, were most likely contemporaneous. Excepting *Spilogale*, *Ursus*, *Erethizon*, and *Cervus* the associated fauna seems to be similar to that at present, the latter three exterminated from southern Indiana by the mid-1800's, and *Spilogale* with an ancient northerly distribution, much as *Neotoma*. This *Neotoma-Ursus*-

Erethizon-Spilogale component commonly occurs together, and can be used locally to recognize a Prehistoric fauna. From faunal evidence the *Neotoma* archaeo-range seems to have been presettlement at minimum, and post-Pleistocene by the lack of true fossil fauna. The presence of *Didelphis*, known to be a late arrival in the Eastern United States (3), sealed in the Carcass Crypt sediments confirms a maximum of at least a few thousand years for at least that deposit.

Illinois and Missouri also have "Recent fossil" finds. Jerry Long cave, Ralls County, Missouri, included a large percentage of *Neotoma*, *Marmota monax*, *Ursus americanus*, *Sylvilagus floridanus*, and *Spilogale* sp., among others. The bones were thought to have accumulated "a few hundred years prior to about 1850". *Neotoma* may inhabit that same area presently, but in less abundance (10). In Meyer cave, Monroe County, Illinois, bones of 535 *Neotoma* were associated with one of the most extensive Recent age faunas ever recovered in the United States, and included *Spilogale* and *Erethizon* where they do not presently occur. *Neotoma* was there 50 miles northwest of its living population (11). *Spilogale* has been recorded from the Modoc Rock Shelter, Randolph County, Illinois, where it does not presently occur, from a level radio-carbon dated at between 4,500 and 2,500 years B.D. (9). Other than the "Recent fossil" finds of Bader and Hall (1) and the author, *Spilogale* has not been definitely recorded in Indiana, paralleling the Illinois situation.

Significantly, by Recent fossil evidence from three Midwest states *Neotoma* has had a distribution much farther north than at present, in Indiana almost 70 miles from the historically recorded Wayndotte *Neotoma*. The distance of the range delineation, age of the Illinois *Spilogale*, inferred ages of other *Neotoma*, and association with *Didelphis* permits a generalized age of a few thousand years, with a wide age span between northerly and southerly sites, to be inferred.

There is a general opinion that following the glaciations climate has changed from cool to warmer than present, and now cooler again. During this warm phase prairies are thought to have expanded easterly, and southern elements northerly (12). Parmalee (11), on the Meyer cave remains, regards *Erethizon* and several other northern forms as belonging to the cool-moist climate of approximately 9,500-7,500 B.C., and *Spilogale* and others as belonging to the warm-dry period of approximately 3,500-1,500 B.C. These forms, including the woodrat, were regarded as becoming expatriated, being unable to adjust to a replacement in vegetation type.

As *Neotoma floridana magister* ranges almost abruptly to the southern borders of Indiana at the Ohio River, yet also ranges up through the cooler Appalachian area, temperature would not seem to play a strong role locally in its distribution. South-central Indiana's vegetation is similar, if not identical, to that of adjacent Kentucky, where *Neotoma* is common. Except for the Ohio River the karst habitat is continuous. Thus, *Neotoma* could probably inhabit Indiana's karst area today. The author holds that there was a widespread, and

effective (*i.e.*, lack of disjunct colonies) depopulation of *Neotoma* in the northerly portion of its range, rather than just an ancient northerly extension, and that with time *Neotoma* would regain its former distribution were it not hampered by its slow immigration (*e.g.*, from cave system to cave system, the Ohio River crossing). The widespread nature of the depopulation would seem to point to climate as the cause; however, with the ecological plasticity of *Neotoma* only an extreme in climate would seem to displace it. The associated fauna indicates that climate was not then at an extreme. Thus, some other ecological factor, perhaps an indirect result of a mild climatic change, would seem to be the cause of the *Neotoma* depopulation.

Acknowledgements

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Notes on Occurrence and Reproduction of Bats In Indiana

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Abstract

A total of 1,907 bats from Indiana was examined, of which over half were big brown bats, *Eptesicus fuscus*, a species which was found to be somewhat active all winter. Most other species were found primarily during April through October. Some *Lasiurus cinereus* appear to hibernate in Indiana. Reproductive information for *Pipistrellus subflavus* and *Eptesicus fuscus* is presented. Most *Eptesicus* had two embryos. Parturition in this species occurs around June 1 in Indiana. Testis size in *Eptesicus* was greatest in August, but at least some individuals appear capable of breeding much of the year. *Lasiurus borealis* produced young about mid-June; juvenile red bats tend to leave their mothers when about 80 millimeters total length and they weigh about 4 to 6 grams. A number of new county distribution records are included in this paper.

Introduction

This paper presents information on seasonal occurrence, reproduction and growth, and distribution for 10 of the 12 species of bats recorded from Indiana (Table 1). These data were collected mostly from 1963 to 1971 during our investigations of Indiana mammals (11, 12, 14, 15, 21). The bats examined were obtained from several sources. Some were shot about wooded or water areas or collected from buildings and caves. Many were collected by citizens of various areas and submitted

TABLE 1. Bats taken, by month, in Indiana during present study.

Species	Month												Total
	J	F	M	A	M	J	J	A	S	O	N	D	
<i>Eptesicus fuscus</i>	34	32	47	73	98	99	265	271	78	44	48	37	1,126
<i>Lasionycteris noctivagans</i>	0	0	0	0	2	2	0	0	3	5	0	0	12
<i>Lasiurus borealis</i>	0	0	0	8	20	98	154	130	35	11	1	0	457
<i>Lasiurus cinereus</i>	1	0	0	1	12	8	18	3	1	2	0	1	47
<i>Myotis austroriparius</i>	0	0	0	0	0	0	0	4	1	0	0	1	6
<i>Myotis keenii</i>	0	0	0	1	1	3	1	5	4	0	2	0	17
<i>Myotis lucifugus</i>	0	0	1	3	8	5	13	51	24	3	3	4	115
<i>Myotis sodalis</i>	0	1	0	4	4	2	4	7	9	4	3	0	38
<i>Nycticeius humeralis</i>	0	0	0	0	3	4	1	3	1	2	1	0	19
<i>Pipistrellus subflavus</i>	0	1	0	2	9	12	8	21	11	1	5	0	70
Total													1,907

to the Indiana Department of Health for rabies examination. We thank Jerry D. Brown for some of the *Eptesicus fuscus* testis measurements.

Results

A total of 1,907 bats from Indiana was examined during the present study (Table 1). More than half (1,126) were big brown bats, *Eptesicus fuscus*, which might indicate this as the most common bat in the state. However, since *Eptesicus* is active during the winter, while *Lasiurus borealis* is not, the months May to September were used for comparison. During those months 811 big brown bats and 437 red bats were taken. Since *Eptesicus* is the common bat in buildings, perhaps citizens are more apt to encounter it than other species. Also, the total for *Eptesicus* is inflated by a few collections of numbers of bats from buildings, 133 from Madison during rabies studies (22) and several other smaller collections. Red bats are solitary and cannot be collected by such methods. We can conclude that these are the two most common bats in Indiana.

Next in abundance would appear to be *Myotis lucifugus* and *Pipistrellus subflavus*. It was surprising to take 47 of the relatively uncommon hoary bats, *Lasiurus cinereus*, most from April to October, but one each in December and January. Perhaps these latter individuals were hibernating in Indiana (20), although this species presumably migrates south for the winter. There are also isolated reports from Indiana and other states that suggest that red bats also may hibernate at this latitude (5).

Except for *Eptesicus* and the two *Lasiurus cinereus*, most bats were taken during the months April through October, except for a few *Myotis* and *Pipistrellus* found in hibernation in caves. It is evident (Table 1) that there is much winter activity in *Eptesicus*, a hibernating species. Many of those taken were active at the time of capture, or were obviously recently active (found in a place where they would have been noticed). It is not known where the majority of the big brown bats spend the winter. Certainly, the large summer population in Indiana cannot be accounted for by the relatively small numbers that hibernate in Indiana caves, and banding records do not yet indicate long-range fall migrations. A group of 17 big brown bats found March 26, 1969, in a barn in Vigo County, was thought to have still been in winter quarters since the bats were torpid, there was no food in their stomachs or intestines, and they were wedged so tightly into tiny crevices that their skins were torn and myobiid mites on their abdomens were crushed when the bats were removed. Seven of the bats were found singly, but groups of 2, 3, and 5 were present. Six of the singles were female; the group of two consisted of two males, and the other groups each contained one male. Examination of the crevices on January 21, 1972, turned up no bats, hence the bats found earlier likely entered the barn after leaving their winter quarters.

The records of *Lasionycteris* are of interest. The previous late spring record for this migrant was May 28 (14), but we have two individuals taken June 1 and June 10 from Lake County.

Reproduction

There is but a single breeding record of *Myotis sodalis* for Indiana (13), but our additional June and July records further substantiate the conclusion that the species breeds there. There is little information on *Pipistrellus* breeding in Indiana. Eight gravid females were shot over a pond in Vigo County and appear to have been the small colony of bats using a nearby barn. The colony disappeared at the time these were shot. Embryos ranged from 11 to 21 mm crown-rump length (avg. 14.3 mm). Six females contained two embryos each; two had one. An additional female, shot in Vigo County on June 30, had recently given birth, as had a female taken July 10 in Jefferson County.

Ovulation in *Eptesicus* occurs about the first week in April in Maryland, and sperms are present in the female genital tract at this time (1); birth occurs about June 1, giving a gestation of about 2 months. Eastern United States populations have two young per litter and western populations one (4, 6, 7). In 1971, three collections of big brown bats were made from a breeding colony in Putnamville, Putnam County, Indiana. On April 14, 7 of 14 females contained no visible embryos; one female appeared to have one tiny embryo; 6 females appeared to have two tiny embryos each (counts based on uterine swellings). Each of 30 females taken May 14 was pregnant. Three each had one embryo; 27 each had two embryos. Embryos averaged 10.7 mm crown-rump length and varied from 4 to 16 mm. Each of 15 females taken May 24 was gravid; two had one embryo each and 13 had two. The average crown-rump length of embryos was 17.3, with a range of 13 to 21 mm. In addition, two females from a Vigo County barn taken April 3, 1963, contained two tiny embryos. Our latest records of embryos in this species are June 1 and 2 (Vigo County) and June 4 (Allen County). The first young of the year appeared in our sample in June. The smallest one, taken June 11, weighed 7.8 g, measured 60 mm in total length, and had a forearm length of 20 mm.

Copulation in *Eptesicus fuscus* occurs primarily in autumn, but may occur also during the winter (7, 16). Copulation of big brown bats has been reported in Indiana caves on November 23, January 8, February 7, and March 12 (10). Phillips (16) found sperm in the reproductive tracts of four of five females taken October 18, in each of four females taken October 29, and in four of five females taken in February and March, in northeastern Kansas. We examined five big brown bats (2 males; 3 females) taken March 26 in Vigo County. The males had living sperm in the epididymus; two of the females had living sperm in their uteri.

Testis size has been found to be greatest in August, but regression occurred by the time of autumn copulation (4, 16). Testes size was tabulated, by month, for Indiana *Eptesicus* (Table 2). In August and September the highest percentage of males in the sample had testes more than 5 mm long and testes size averaged greatest in those months. This indicates that the largest amount of breeding activity takes place in August and September, but judging from testis size males may be capable of breeding much of the year.

TABLE 2. *Testes measurements in mm, by month, of male Eptesicus fuscus and Lasiurus borealis, Indiana.*

	Number	No. with Testis ≥ 5	% with Testis ≥ 5	Avg. Testis Length	Avg. Testis Width
<i>Eptesicus fuscus</i>					
Jan.	13	7	53.8	4.42	2.19
Feb.	14	8	50.0	4.81	2.56
Mar.	15	9	60.0	5.37	2.40
Apr.	22	12	54.5	4.76	2.11
May	29	12	41.4	4.77	2.41
June	24	17	70.8	5.37	2.55
July	54	41	75.9	5.87	3.04
Aug.	117	98	83.8	6.88	3.72
Sept.	32	31	96.9	7.06	3.75
Oct.	14	7	50.0	4.43	2.68
Nov.	18	11	61.1	5.48	2.53
Dec.	14	8	57.1	5.04	2.29
<i>Lasiurus borealis</i>					
Apr.	2			3.50	2.50
May	3			3.30	1.50
June	4			4.13	2.25
July	22			4.43	2.55
Aug.	13			4.99	3.15
Sept.	9			5.06	2.89
Oct.	2			3.00	2.00
Nov.	1			4.00	2.00

Few red bats were taken that could be classed as adult males. From April through June, before young males entered the population, only 11 of 56 (19.6%) adult red bats were males. A similar sex distribution has been found in Kansas (3, 8).

Of 13 gravid female red bats examined, 2 each had 2 embryos, 7 had 3, 2 had 4, 1 had 5, and 1 had embryos destroyed by shot. An average of 3.2 embryos among 44 pregnant females has been reported (1). The earliest embryos found by us were in a female collected April 30; she contained 4 measuring 1.8 mm in diameter. The latest date was June 19 (3 embryos 10 mm crown-rump length). Crown-rump measurements were made on other embryos as follows: May 9 (10 mm); May 17 (10 mm); May 21 (9 mm); May 26 (6 mm); May 26 (12 mm); May 26 (10 mm); May 31 (12 mm); June 5 (15 mm); June 14 (22 mm). The three embryos found June 14 were probably near term; the eyes were visible as dark spots and the milk teeth had erupted. Standard measurements of these young were total length 44, 44, 41 mm; tail length 15, 12, 12 mm; hind foot length 6, 6, 6 mm. The one male had testes 1 by 1.5 mm. Young taken with adult females on June 6, 14 and 17 were of

similar size and were possibly newborn. Hamilton (6) stated that young were born about mid-June, while in Kansas and southern Illinois (8, 9) parturition occurs in late May and early June. A female taken April 19 appeared to have given birth, judging from the expanded and flaccid condition of the uterus. This would seem to be an unusually early date. Another female taken May 3 had four placental scars. Twenty females that had given birth were collected between June 20 and July 20. It appears that most females give birth about mid-June in Indiana.

The largest young taken with a female (3 young, July 14) measured 79, 79, and 81 mm in total length and weighed 6.4, 7.4 and 7.6 g. Young only slightly smaller were taken with four other females on June 20, June 23 and two on July 1. One young, taken with a female on June 15, weighed 4.4 g. and was 64 mm total length (Table 3). The stomach of this young contained 60 chironomid and 40% Coleoptera remains. Two other large young taken with females had traces of vegetation and milk in their stomachs.

TABLE 3. *Size variation in young red bats taken with females in Indiana.*

Date Found	No. Juv.	No. Females	Total Length (mm)	Tail Length (mm)	Hind Foot (mm)	Weight (g)	Forearm (mm)
June 15	4	1	63-65	20-21	6-6.5	4.2-4.4	
June 20-23	16	5	50-77	14-29	6-8	2.2-7.5	24-36 ¹
June 30-July	12	3	44-77	13-29	5-8	3.6-5.0 ²	17-34
July 14	3	1	79-81	29.32	6-7	6.4-7.6	31-36

¹Forearm data not available on smallest two litters.

²Smallest not weighed.

The 29 juvenile red bats less than 80 mm in total length and taken without accompanying females are of interest. We do not know whether some of this sample had been weaned, but suspect that most (or all) had merely become separated from their mothers. All of the above young were collected in late June and July (July 31 the latest) and all that had any food in their stomachs contained milk; two of them (with forearms of 31 and 27 mm) contained parts of ants and other insects in their intestines. Three large young found on June 30 had milk in their stomachs and intestines; the largest of this trio measured 91 mm in total length, had a forearm of 38 mm, and weighed 7.5 g. Judging from the growth rate of other young examined, this litter must have been born in early May. Two lone immatures taken July 28 and August 11 contained only insect remains, thus were evidently independent of their mothers. These bats had total lengths of 85 and 86 and forearms of 36 and 39 mm, respectively. It would appear that juvenile red bats tend to become independent of their mothers when about 80 mm in total length and weighing from 4 to 6 gm.

None of 25 adult female red bats shot in June and July during our study were carrying young. Despite various records (6, 7) of females

carrying their young on foraging flights, we do not feel that this occurs regularly. Females with attached young may be frightened from their roosts, thus fly off carrying the young (if the young are not too large) or be forced to the ground by the weight of large, attached young. More data are required on this.

Hamilton (6) collected female red bats with quantities of sperm in their uteri the first week in August and stated that the species mates in August, while other authors (1, 7) thought mating probably takes place in August and September. Males taken in Indiana have the largest testes in August and September (Table 2) and although testes size alone is not necessarily proof of reproductive ability, it does suggest that this is the season of mating.

Female hoary bats with two young each were taken May 23 (Johnson County) and June 19 (Vigo County). The May young had total lengths of 79 and 80, forearm lengths of 36 and 37, and one weighed 7.1 g. Comparable data for the young taken June 19 were total lengths 100, 102; forearm lengths 42, 44; and weights 13.2 and 13.1 g. A lactating female captured May 28 gave birth to two young that day. Whether this was normal parturition is unknown, but there are numerous instances in the literature of births shortly after capture, some of which may be cases of induced abortion brought on by conditions of captivity. Caution should be used in considering such cases as parturition dates. Young are probably born in Indiana in late May or early June. Two small adult females (total length 105, 107, forearm 51, 52) taken in late May, but showing no evidence of reproductive activity, suggest, on very limited data, that females might not breed their first year.

Three evening bats each with two embryos were examined, one taken May 20 in Monroe County, one May 26 from Tippecanoe County, and one June 27 from Marshall County.

Distribution records

New county distribution records for bats in Indiana follow:

Myotis lucifugus: Clinton, Gibson, Hancock, Howard, Madison, Tipton, Union and Vanderburgh.

M. keenii: Johnson, Vanderburgh.

M. sodalis: Dubois, Hancock, Hendricks, Jefferson, Johnson, Marion, Morgan, Vanderburgh.

Lasionycteris noctivagans: Hendricks, Howard, Lake, Vanderburgh

Pipistrellus subflavus: Gibson, Johnson, Morgan, Spencer, Vanderburgh, and Wells. (The latter represents the most northern locality in Indiana where the pipistrelle has been recorded and is some 80 miles north of Wayne County, the closest previously known station. This was the only individual taken north of Marion County.)

Eptesicus fuscus: Bartholomew, Clark, Floyd, Hancock, Hendricks, Howard, Jasper, Knox, Lake, Martin, Miami, Morgan, Newton, Starke, and Sullivan.

Lasiurus borealis: Adams, Bartholomew, Brown, Gibson, Greene, Hancock, Hendricks, Jasper, Johnson, Madison, Noble, Rush, Shelby, Sullivan, Vanderburgh, and White.

L. cinereus: Clay, Elkhart, Johnson, Morgan.

Nycticeius humeralis: Carroll, Clinton, Gibson, Jefferson, Johnson, Marshall, Monroe, and Vanderburgh.

Discussion

It is apparent that winter bat activity occurs in Indiana during the period when we generally assume that bats (especially *Eptesicus fuscus*, various species of *Myotis*, and *Pipistrellus subflavus*) are hibernating. Whether such activity is usual requires more investigation; it would seem that much of it, in the case of *Eptesicus* particularly, is normal. Urine in the bladder may cause bats to wake periodically (19). While the need for water may be responsible for winter movements (16), Rysgaard (17) felt that some food was necessary in winter, for he found chitin in the mouths of some bats; he also found that *Eptesicus fuscus* drank water in winter. Other authors also feel that winter activity is for feeding (1, 18). In Indiana, winter movement appears not to be related to feeding, based on stomach analyses of winter-taken specimens (Whitaker, unpublished data). Big brown bats have been kept for six months at 4.5°C without food with no ill effects (2).

Delayed fertilization is presumably advantageous to bats, allowing them to mate in the fall when they are in good condition with plenty of food, thus avoiding an excess use of energy during early spring when energy may be at a premium. This advantage would seem to be negated, at least in part, if bats mate in winter, as appears to be the case in *Eptesicus fuscus* (10) and in *Myotis lucifugus* (23). Additional winter activity would further drain this energy and would seem to be strongly selected against, if energy is at a premium. It may well be that bats are physiologically adapted so that they can easily survive the winter on stored fat with energy to spare. Possibly, winter activity facilitates mating, ensuring that a high percentage of females become pregnant. This would be an advantage since the reproductive potential of bats is low (one litter per year and one to two young per litter in most species). Delayed fertilization may have evolved along with winter mating, allowing young to be born at the optimum time. Delayed fertilization might also be a mechanism by which bats take advantage of chance matings over extended periods of time.

It also appears that *Lasiurus cinereus* and *Lasiurus borealis*, both considered migrant species that leave the latitude of Indiana in winter, may actually remain in winter. Possibly the portion of the total population that behave in this fashion is small, but we need further data. The fact that *Lasiurus borealis* will appear and fly about during periodic warm periods in mid-winter in Indiana indicates that these animals probably were nearby.

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Rodent Seed Supply and Burrows of *Peromyscus* in Cultivated Fields

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Abstract

Food supply, in the form of seeds, was investigated in corn and soybean fields in Vigo County, Indiana, to determine what effect availability of this food had upon the distribution and abundance of prairie deer mice, *Peromyscus maniculatus bairdii*, and house mice, *Mus musculus*. Food was abundant in all study areas and did not adversely affect the distribution or abundance of either species.

Twelve burrows constructed by *P. m. bairdii* were examined and sketches of two of these are presented. The burrows averaged 16.0 feet long, 4.1 inches deep, and had 6.0 branches with only one opening per burrow. The branch nearest the opening commonly had a deep V-shaped tunnel which may serve as shelter during periods when the ground is being worked. Chambers containing feces, nesting material, or food caches of seeds were frequently found.

Introduction

Food supply has often been thought of as an important factor limiting distribution and abundance of animals (1, 5), but there are conflicting reports (2, 3, 4) which indicate that food is not an important factor limiting the distribution of *Peromyscus*. The present paper is an attempt to determine if food, in the form of seeds, is a critical factor influencing the distribution and/or abundance of two common rodents, *Peromyscus maniculatus bairdii* and *Mus musculus*, in cultivated habitats of Vigo County, Indiana.

Peromyscus maniculatus bairdii constructs burrows, but there are apparently no previous reports describing the burrows. Burrows examined during this study are described.

Study Areas

Three separate areas totaling approximately 2,800 acres were chosen for study, with corn and soybeans being the most prevalent cultivated crops. Area (Secs. 21, 22, 27, 28, T13N, R9W), 1,100 acres, 3 miles north of Terre Haute, Indiana, was adjacent to the Wabash River and was part of the Wabash Floodplain. Area 2 (Secs. 25, 36, T13N, R9W, Secs. 29, 30, 31, T13N, R8W), 800 acres on the Wabash River terrace, was approximately 2 miles northeast of North Terre Haute, and was composed of 4 separate fields. Area 3 (Secs. 3, 4, 5, 8, 10, T12N, R8W), 900 acres on an upland site, was 1 mile northwest of Seelyville, Indiana, and contained 4 separate fields.

Methods

Maps of each area were constructed using aerial photographs and vegetation maps. A scale of 1:4800 was used. Each map was divided into sampling plots of 76 by 76 feet numbered in rows from west to east

and with the first row to the north. Ten plots per area per trapping period were randomly selected for study. Plots not in cultivated fields were omitted. In each plot, 25 traps were set in 5 lines of 5 traps, with 15 feet between each trap and 7.5 feet between the outer traps and the edge of the plot.

Mammals for this study were collected during four trapping periods: July 1970; August 1970; September-October 1970; and December-January 1970-71. All specimens were taken by means of standard snap-back mousetraps baited with peanut butter. Traps in each area were checked each trapping period for 4 consecutive days, resulting in 12,000 trap-nights for the entire study.

A square foot of soil, sampled to a depth of one-half inch, was collected in each study plot for seed analysis. Seeds were isolated according to a technique described by Malone (6). The seeds from each sample were placed in marked Petri dishes, dried in an oven, identified by comparison to known samples, and counted.

A Chi-square goodness-of-fit test was used to determine significance of differences in the numbers of mice taken per trapping period. A completely randomized design of analysis of variance was used to determine significance of: 1) differences in the numbers of mice per species per study area, 2) differences in the total number of seeds per sampling period, 3) differences in the total number of seeds per study area. A t-test was used in cases where a significant F value from Analysis of Variance was obtained.

Twelve burrows were examined in Area 2 during February and March 1970. Attempts at locating burrows in Areas 1 and 3 were unsuccessful due to the absence of snow and the onset of cultivation. Traps placed near the burrow openings yielded only *Peromyscus maniculatus bairdii*; thus the burrows were presumed to be those of *P. m. bairdii*.

Results and Discussion

Rodent, Population and Seed Supply

During this study 414 prairie deermice (*Peromyscus maniculatus bairdii*), 252 house mice (*Mus musculus*), 22 woodland deermice (*Peromyscus leucopus*), 3 jumping mice (*Zapus hudsonius*), 2 prairie voles (*Microtus ochrogaster*), and 1 rat (*Rattus norvegicus*) were taken. The totals for *P. m. bairdii* and *Mus musculus* were divided into numbers of mice per study area and per trapping period. There were no significant differences between the average number of *M. musculus* per plot in the floodplain (2.0), river terrace (2.4), and upland area (2.0) ($F = 0.29, 119 \text{ df}$), indicating similar populations in the 3 areas. Average numbers of *P. m. bairdii* per plot in the floodplain (6.0), river terrace (2.7), and upland area (1.3) were significantly different ($F = 28.96, 119 \text{ df}$). Study plots in the floodplain area had more *P. m. bairdii* than those in the river terrace area ($T = 5.04, 78 \text{ df}$), while those in the river terrace area had more *P. m. bairdii* than those in the upland area ($T = 2.44, 78 \text{ df}$).

TABLE 1. Numbers of seeds per sampling period and per study area taken in Vigo County, Indiana, 1970-71 ($\bar{X}$ = average number of seed per sample.)

Seed Species	Average Number of Seeds per Area				
	July 1970	$\bar{X}$	Aug. 1970	Sept.-Oct. 1970	Dec-Jan. 1970-71
<i>Chenopodium</i> and <i>Amaranthus</i>	1,495	49.8	1,860	1,178	1,388
Unid. spores	462	15.4	411	430	326
Unid. mustard	397	13.2	336	268	253
<i>Setaria faberii</i>	252	8.4	240	1,044	587
Others	382	12.8	370	297	429
Totals	2,988	99.6	3,217	3,217	2,983
				107.2	99.4

Seed Species	Average Number of Seeds per Sampling Period				
	Area 1	$\bar{X}$	Area 2	Area 3	Total
<i>Chenopodium</i> and <i>Amaranthus</i>	442	11.1	4,521	958	5,921
Unid. spores	819	20.5	403	407	1,629
Unid. mustard	35	0.9	322	897	1,254
<i>Setaria faberii</i>	135	3.4	1,369	619	2,123
Others	477	11.8	480	521	1,478
Totals	1,908	47.7	7,095	3,402	12,405
			177.4	85.1	103.4

Average numbers of *P. m. bairdii* per plot in July 1970 (3.0), August 1970 (4.4), September-October 1970 (3.1), and December-January 1970-71 (3.3), indicated similar populations during all 4 trapping periods ($F = 0.96$, 119 df). Average numbers of *M. musculus* per plot in July 1970 (1.9), August 1970 (1.9), September-October 1970 (3.5), and December-January 1970-71 (1.1), indicated an increase in late autumn followed by a decrease in winter ($F = 4.29$, 119 df). These results agree with those of Whitaker (7), who found that *P. m. bairdii* remained at roughly similar population levels throughout the year while *Mus* moved out of areas when ground cover decreased.

Information on the number of seeds taken was summarized according to sampling period and study area (Table 1). Species which had an average number of seeds per sample greater than 5.0 are listed. Other species less frequently found were *Datura stramonium*, *Cerastium vulgatum*, *Aster* sp., *Polygonum* sp., *P. pennsylvanicum*, *Ambrosia trifida*, *Brassica kaber*, *Hypericum perforatum*, *Rumex crispus*, *Glycine max*, and *Zea mays*.

There were no significant differences in the mean number of seeds per sample per sampling period ($F = 0.09$, 119 df). There were, however, significant differences in the mean numbers of seeds per sample per study area ($F = 51.60$, 119 df), with the extremes in Area 1 of 47.7 seeds versus Area 2 with 177.4 seeds. Significant differences were also found in the mean numbers of seeds per sample between Areas 2 and 3 ($T = 5.88$, 78 df).

If food in the form of seeds is a critical factor, mouse numbers should be influenced by seed availability. Since no significant differences were found in the mean numbers of seeds per sampling period (99.6, 107.2, 107.2, 99.4), while significant differences were found in the number of *M. musculus* per sampling period, seeds as a food source apparently did not limit *M. musculus* numbers. The floodplain area had the fewest seeds per sample (47.7), yet this area contained the most *P. m. bairdii* per plot (6.0). No significant differences were found in the number of *M. musculus* per plot per area ($F = 0.29$, 119 df).

The number of seeds per sample apparently did not influence the abundance of either *P. m. bairdii* or *M. musculus*.

Burrow Studies

Burrows were most easily found when snow cover was present. The burrows constructed by *P. m. bairdii* appeared to offer an adequate habitat for living, feeding, and nesting. Two burrows are diagrammed (Fig. 1).

The 12 burrows averaged 16.0 feet in length (range, 5 to 35 feet), and 4.1 inches in depth (range, 1 to 12 inches). The average burrow had 6.0 branches (range, 1 to 12), and was 6.3 inches at its deepest point. Burrows ranged from 0.75 to 1.50 inches in diameter. Only one opening was found for each burrow. All of the burrows had a drop of 2 to 3 inches from the opening, then a relatively straight tunnel of 4 to 6

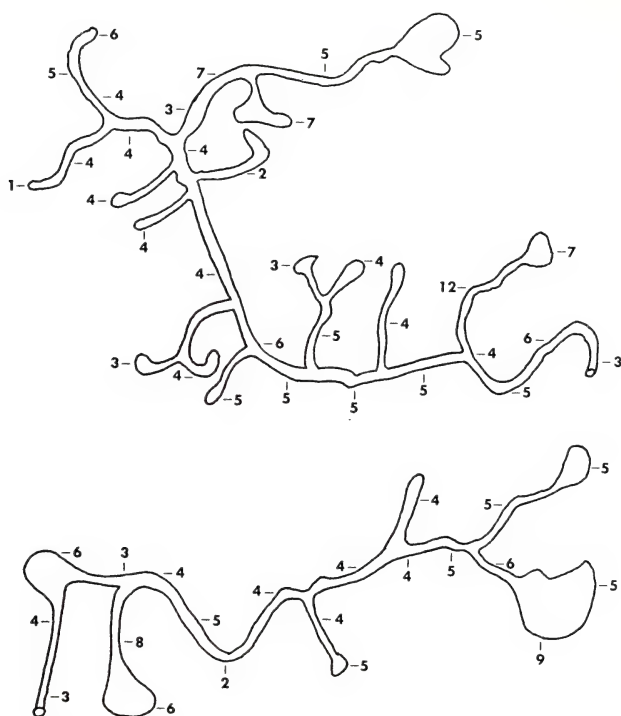


FIGURE 1. Top view of two *Peromyscus maniculatus bairdii* burrows in Vigo County, Indiana. Numbers indicate depth of burrows in inches. Linear scale: $\frac{1}{2}$ inch = 2 feet.

inches before the first turn. The deepest point in the burrow was frequently found in the branch nearest the opening. This branch commonly had a steep decline of 30 to 40°, then an abrupt incline at the same angle which opened into a chamber. Three of the burrows had caches of uneaten seeds, mainly *Setaria*, at this chamber site, indicating food storage by this species. The deep, V-shaped tunnel may serve as shelter during periods when the ground is being worked. Most of the burrow would be destroyed but this area would remain. Many of the other tunnels had similar chambers, but without the steep slope or incline before them. In 6 of the burrows, feces were found in these chambers. Eight burrows terminated in a large chamber and three of these contained nesting material of finely chewed grass, straw, leaves, and paper. In all cases, the main tunnel was free of debris. These findings indicate a high degree of burrow utilization by *P. m. bairdii*.

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Effect of Aging on Ability to Acclimate to Chronic Hypoxia of Simulated High Altitude¹

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Abstract

Young (1 month old) and old adult (13 months old) rats were subjected to a simulated high altitude (23,000 feet for 5 weeks) environment. The anoxic resistance of isolated right ventricles, right ventricular weights and hematocrit values were then measured in both sea level control and altitude-acclimated animals from both age groups. This research demonstrates that the hematocrit cannot be increased in older individuals to such an extent as in young individuals and that the right ventricular hypertrophic response to hypoxia is markedly decreased in the older animal. Older animals were shown to have increased their myocardial anaerobic competency as a result of the aging process. Young animals can be forced to increase their anaerobic competency by chronic exposure to hypoxia, but the old myocardium cannot be forced to induce further anaerobic competency by such hypoxia exposure.

Introduction

It has been well established that several parameters of the cardiovascular system are modified as a result of chronic exposure to the hypoxia of high altitude (9, 11, 16, 17, 29). Increased hematocrit, or polycythemia, has been shown to be a meaningful high altitude adaptation by several researchers, both on the basis of increasing the oxygen-carrying capacity of the blood (8, 11, 29) and of increasing the capacity of the animal to perform work under hypoxic conditions (7, 21). This enhanced erythropoietic activity has been shown to be mediated by a hormonal factor which produces bone marrow hyperplasia accompanied by an augmented rate of iron turnover (18, 19).

Right ventricular hypertrophy has been shown to consistently accompany high altitude exposure, whether one employs laboratory animals acclimated to simulated high altitude or natively acclimatized high altitude species (9, 10, 31, 32). This right ventricular hypertrophy is reflected in the EKG as a right-axis deviation (20), and it has been shown to result from the pulmonary hypertension which accompanies the hypoxia of high altitude acclimation (1, 9, 20, 27.)

In addition to these anatomical changes which accompany altitude acclimation, several investigators have demonstrated an increased

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anaerobic capacity of the brain (2), striated muscle (2, 13, 17) and cardiac muscle (14, 15, 16, 17, 22), as well as an increased percentage recovery of cardiac muscle after an anoxic stress (23).

By examining these three parameters—hematocrit, heart weight and myocardial anoxic resistance—in sea level rats and in altitude-acclimated rats whose ages ranged from young to old adult it would be possible to show whether or not the aging process had altered the animal's ability to develop these cardiovascular adaptations in response to chronic hypoxia.

Materials and Methods

Animals

Male albino rats (Sprague-Dawley) were purchased from Purina Laboratory Animals, Vincentown, N. J. These rats were fed a special thyroxine-free synthetic diet (Vitamin B complex test diet, with salt mixture USP XIV substituted for Salt Mixture #2, completely vitamin and mineral fortified and pelleted for rats) purchased from Nutritional Biochemicals and tap water *ad libitum*. The animals were maintained on this test diet for 2 weeks before experimentation was begun, and this diet was administered for the duration of the experiment. Animals employed in the study were either 1 month or 13 months old. These represent respectively young and older mature rats.

Lighting, Temperature and Altitude

Animals were maintained in the laboratory at $25\pm1^{\circ}\text{C}$, and the fluorescent lighting provided 12 hours of light and 12 hours of darkness daily. The altitude-exposed animals were maintained in altitude chambers at a simulated 23,000 feet above sea level (14, 15). The high-altitude animals were acclimated for 2 days at 7,000 feet, 3 days at 10,000 feet, 3 days at 17,500 feet, 3 days at 20,000 feet, and for the remaining 5 weeks at 23,000 feet. These altitude-exposed animals remained at 23,000 feet for 72-hour intervals; they were then returned to sea level for 1 hour to allow for cleaning, disinfecting and feeding and were then depressurized to 23,000 feet for another 72-hour interval. This depressurization was accomplished gradually over an hour period so as to avoid aeroembolism. The "sea level controls" were maintained in the same laboratory at a Philadelphia, Pennsylvania, altitude of approximately 150 feet above sea level.

Hematocrit Determinations

The animals were anesthetized with ether, guillotined, and heparinized micro-hematocrit tubes were filled with blood from the severed carotids and were spun down on a clinical centrifuge to determine each animal's hematocrit value.

Right Ventricular Function Test

After blood was collected for hematocrit determinations, the rat was restrained on a dissecting board, an incision was made from the

ventral mid-line of the neck to the xyphoid process, and the ribs and musculature on the left side of the sternum were severed and separated with a wound retractor. The pulsating heart was removed and placed in a beaker of well-aerated (95% O_2 - 5% CO_2) Krebs-Ringer's bicarbonate solution to remove the excess blood. The right ventricle was dissected away (Fig. 1) and was rapidly transferred into the Krebs-Ringer's bicarbonate-filled muscle chamber (Fig. 2) which was aerated by the 95% O_2 - 5% CO_2 mixture. After an initial stabilization period of 30 minutes, the aerating mixture was changed to 95% N_2 - 5% CO_2 as an anoxic test. This anoxic test was applied for a 20-min period, and then the 95% O_2 -5% CO_2 mixture was re-introduced for a 20-min recovery period. During the entire test the isolated right ventricle was kept at 29° C and was driven at a supra-maximal stimulus strength (10 volts, 7 msec) at a frequency of one stimulus per second. The apical end of the excised ventricle (Fig. 1) was pinned and attached to an isometric strain gauge (Stratham Instruments). The output signal from the strain gauge was recorded on an Electronics for Medicine recording system, and the resting tension of the excised ventricle was set at 1 g.

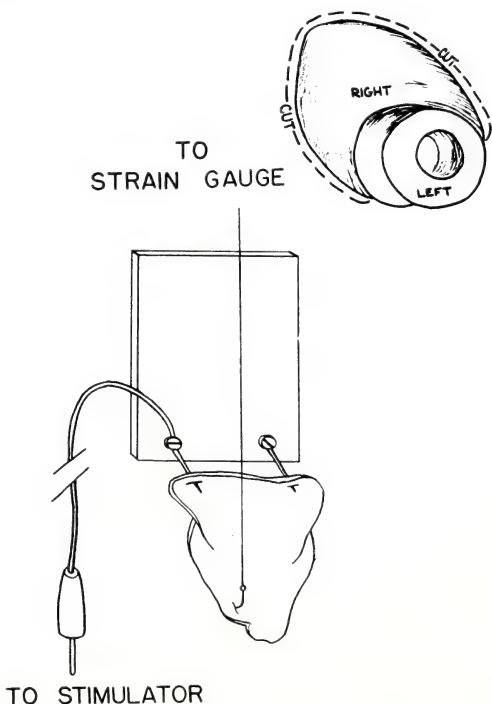


FIGURE 1. *Right ventricular procurement and fixation to muscle chamber and strain gauge.*

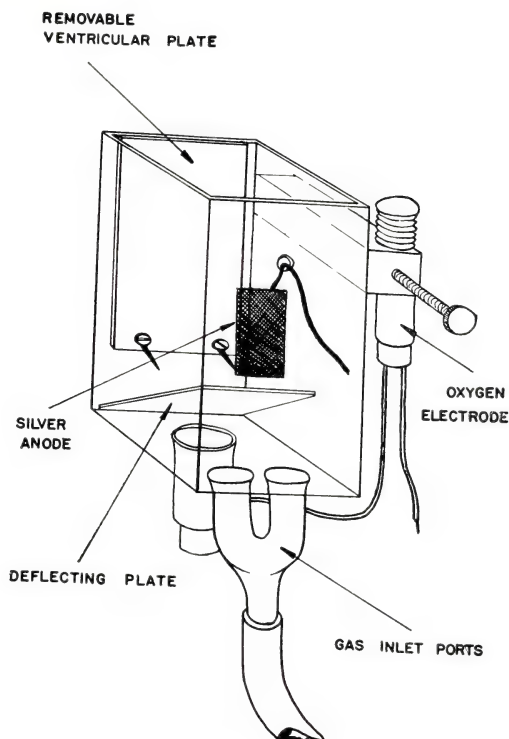


FIGURE 2. Plexiglass muscle chamber used to conduct anoxic resistance tests.

Heart Weight

At the conclusion of each experiment, the excised right ventricle was blotted and weighed on a Mettler H 20 T (Fisher Scientific) and was analyzed on the basis of 100 g of body weight.

Results

Hearts were analyzed on the basis of right ventricular weight per 100 g of body weight. Right ventricular weights were increased in both young and old adult rats by altitude exposure (Fig. 3). However, while altitude acclimation induces a 171% increase in right ventricular weight in the young (2 months of age) group of rats, the same hypoxic exposure elicits only a 69% increase of the same parameter in the older (14 months of age) group.

Hematocrit values were determined and compared among both age groups at sea level and after altitude exposure (Fig. 4). When compared to sea level controls of the same age, altitude acclimation elicits a 92% increase in the hematocrit value of the young group, but the older group shows only a 67% increase following the same chronic hypoxic exposure. The maximal hematocrit values achieved by the two groups

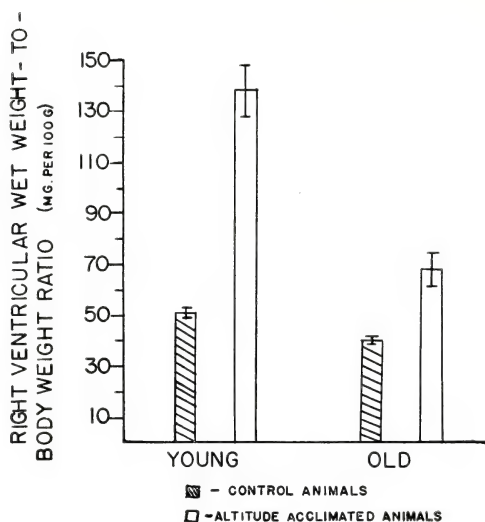


FIGURE 3. Relationship between ventricular weight and ambient pO_2 in young and older mature rats. Vertical line indicates mean ± 1 S. D.

after altitude acclimation, however, are not significantly different ($P > 0.05$).

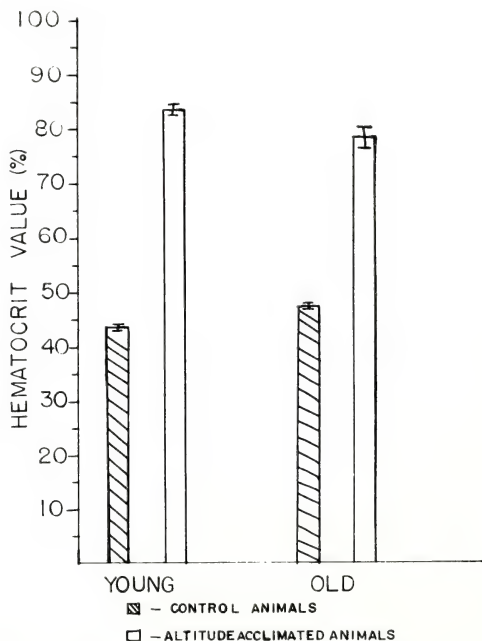


FIGURE 4. Hematocrit changes in young and old rats in response to chronic hypoxia. Vertical line indicates mean ± 1 S. D.

TABLE 1. *Effect of aging on certain cardiovascular adaptations to chronic hypoxia of high altitude.*

Age Group	Treatment	n	Hematocrit	S.E.	Weight (mg/100 g Body Wt.)	Right Ventricular	S.E.
Old Adult (14 mo.)	Sea-level Control	13	46.9	0.6	40.0	40.0	1.5
Old Adult (14 mo.)	23,000 ft.-5 wks.	6	78.5	1.7	67.9	67.9	6.4
Young (2 mo.)	Sea-level Control	11	43.2	0.4	51.2	51.2	1.8
Young (2 mo.)	23,000 ft.-5 wks.	10	82.9	1.2	138.5	138.5	10.2

Percentage Preanoxic Contractility Remaining After							
Age Group	120 anoxic contractions (2 min)	n	S.E.	480 anoxic contractions (8 min)	S.E.	960 recovery contractions (16 min)	S.E.
Old Adult (S.L.C.) (14 mo.)	79.5	13	2.7	25.1	3.5	26.4	1.7
Old Adult (Alt.) (14 mo.)	79.6	6	4.1	24.9	5.9	38.5	5.1
Young (2 mo.) (S.L.C.)	69.0	11	2.2	7.7	0.9	35.3	1.3
Young (2 mo.) (Alt.)	67.3	10	2.4	25.0	1.5	43.0	4.4

To compare both early and late responses to anoxia, contractile strengths were measured after both 120 and 480 anoxic contractions and were expressed as a percentage of the control preanoxic strength (Table 1); after the 20-min anoxic period had elapsed, the gassing mixture was switched back to 95% O₂ - 5% CO₂, and the recovery was measured after 960 additional contractions in the aerobic recovery period. The recovery was then expressed as a percentage of the control preanoxic strength (Table 1). It can be seen that whereas altitude acclimation afforded the young animals no increase in preanoxic contractility after 2 min (120 anoxic contractions) when compared to young sea level controls and a 225% increase in the same parameter after 8 min into the anoxic period (480 contractions), the old altitude-acclimated animals showed no increase in anoxic resistance when compared to old sea level controls after either 2 min or 8 min. On the other hand, when compared to young controls, the old controls showed a 15% added anoxic resistance after 2 min into anoxia and a 226% added anoxic resistance after 8 min of anoxia. Thus, while old sea level-control rats show a greater anoxic resistance than do young sea level-control animals, the anaerobic competency of the young ventricle can be increased to the same level as that of the old ventricle by chronic altitude acclimation. The older ventricle, however, cannot be forced to increase its anaerobic competency by altitude exposure.

Both age groups were compared after 16 minutes of recovery with the 95% O₂ - 5% CO₂ mixture (Table 1). While the young and old acclimated animals and the young sea level control animals have recovered 43%, 39% and, respectively, of their preanoxic contractility values, the old sea level control animals have regained only 26% of their original control value.

Discussion

While our results indicate that older animals do not show as great a percentage change in their hematocrit as do young animals in response to the same hypoxic stress, both age groups were able to reach the same maximal hematocrit value in response to the hypoxic stress. This finding contrasts with a previous study on humans by Dill (5, 6), in which he reports that both the rate and equilibrium value of hematopoiesis during altitude exposure may be depressed by the aging process. These studies, however, involved only a few subjects who were tested after only a 2-week sojourn at 3,000 m. Our animals, on the other hand, remained at 7,012 m for 5 weeks.

The fact that the older right ventricle does not show the extent of hypertrophy as does the young right ventricle in response to the hypoxia of chronic altitude exposure may be due to several factors. It has been established that the right ventricular hypertrophy of the altitude-acclimated animal results from pulmonary hypertension which accompanies exposure to hypoxia (1, 9, 20, 27). This implies, however, that: 1) the lung vasculature must be reactive to the hypoxia to which it is exposed, and 2) the right ventricle is capable of responding to this extra work load by increasing its muscle mass. The first of the require-

ments is diminished by the aging process, for active microvascular responses are both more limited and slower in older animals (3, 33). The second requirement is modified by the facts that in the aging muscle there is a decrease in the concentration of DNA and RNA, there is a decreased uptake of amino acids by the older muscle cell and there is a decrease in essential transaminases required for protein synthetic reactions (3). This decreased ability of the aging myocardium to respond to greater work loads by hypertrophy has been noted in the dilated ventricles of old rats (28). In addition, Poupa postulates that while young animals increase their cardiac muscle mass in response to increased work loads primarily by increasing the number of myocardial cells (hyperplasia), older animals respond to the added work load by increasing the size of pre-existing myocardial cells (24, 26). Furthermore, according to many researchers, there is an actual disappearance of patent vasculature in the myocardium of the old animal with aging (24, 25, 26, 30). As Poupa points out, "In young animals, overloading of the heart leads to an accelerated growth of both structures (cardiac cells and capillaries), while in adult animals growth is confined to cardiac cells only" (24).

Thus, in comparison with the younger myocardium during hypoxic exposure, the aging heart may be given less of an increased work load as a result of less reactive pulmonary vasculature, the actual protein synthetic reactions required to effect an increase in ventricular mass are depressed by aging, and the older myocardium cannot increase the oxygen-supplying structures to the muscle cells while increasing the oxygen-consuming structures as can the young myocardium. All of these aging changes may contribute to the fact that the older myocardium responds with far less increase in muscle mass than does the young myocardium to a given hypoxic stress.

Finally, this research indicates that while the aging myocardium possesses a greater anaerobic competency than does the young myocardium, the anoxic resistance of the older myocardium cannot be increased by exposure to chronic hypoxia while that of the young myocardium is greatly increased. This finding supports a recent observation of McGrath *et al.* (unpublished, personal communication) that the older myocardium cannot be forced to increase its anaerobic competency by chronic hypoxic exposure. What might be responsible for the increased anaerobic competency in the older sea level-control animal, and why could it be increased no further by hypoxic acclimation? It has been demonstrated that the oxidative metabolism of the myocardium decreases greatly with age with little or no deterioration of the glycolytic and dehydrogenating activity (3). Since ATP levels are maintained at the normal level in the myocardium of the aging animal and since the ATP-ase activity of the myosin does not change with age (3), the demand of ATP for myocardial contraction in senility must remain unchanged. Since myocardial aerobic metabolism has decreased with aging, some alternative anaerobic metabolic mechanism must be employed to a greater degree if energy requirements are to be met—*i.e.*,

if some enzymatic oxidative activities are decreased by the aging process, others must be increased compensatorily if energy requirements remain constant and are satisfied (3). Such a mechanism has been elucidated in the aging erythrocyte, such that as its aerobic competency decreases with age, its anaerobic competency increases so that the concentration of ATP remains constant (3). The search for such a mechanism in the aging myocardial cell continues, however, for the pentose phosphate pathway appears to remain relatively unchanged (3) and the total LDH activity reaches its peak and then begins to decrease at 30 weeks of age as do the H_4 and M_6 isoenzymes (12).

If the myocardial cell in the older animal is employing its anerobic processes to a greater degree than is the young myocardial cell in compensation for a decreased aerobic capacity, one should expect to see evidence of a heightened anaerobic capacity in the older cell. Indeed, our results indicate the presence of such an increased anaerobic competency as added anoxic resistance in the older myocardium. Also, since the diffusion distance for oxygen from the coronary capillaries to the myocardial cell is increased in the aging ventricle due to hypertrophy of the myocardial cells and to the disappearance of patent capillaries (24, 25, 26, 30), local cellular hypoxia may also have further induced the development of a greater anaerobic competence in the older heart (4). From the results of this experiment, it would appear that the young myocardium can be influenced by chronic hypoxia to increase its anaerobic competence to the maximal level at which the older sea level controls are already operating. Under these experimental conditions, the old adult cannot be stimulated to increase its anaerobic capacity above this maximal level.

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Preferences for Natural and Artificial Sweeteners in Heat-Stressed Chicks of Different Ages¹

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Abstract

Chicks are capable of discriminating various taste stimuli and their aversions for cyclamate and preferences for glucose are similar to those reported for various rodents. The age differences found here deserve further investigation as does the effect of heat-stress during incubation on preference behavior.

Introduction

Preferences for various sugars are found throughout the animal kingdom, particularly among mammals. Until recently, reports of sugar preference in chickens had been sporadic and contradictory (4, 5). However, Wagner and Gunther (9) have shown that new-born chicks have definite preferences for glucose and sucrose over water and gradually develop an aversion for lactose. Higher incubation temperatures (heat-stress) failed to result in marked sugar preference differences although heat-stress has been shown to produce marked differences in other behaviors such as discrimination learning, reversal learning, frequency of pecking, and color preferences (1, 2, 3).

Research on sweeteners other than sugars (sweet as judged by humans and accepted as rewards by rodents) as well as the well-publicized ban on cyclamates has spurred the study of preferences for various substances such as saccharin and cyclamate. Wagner (6, 7) and Wagner and Gunther (9) have found several rodent species which prefer saccharin and avoid cyclamate. Whether this cyclamate aversion is because of its taste (or off-taste) or to the suspected toxicity of cyclamates has not been resolved.

The purpose of the present study was to examine preferences for saccharin, cyclamate, glucose, and water in normal and heat-stressed chicks at various ages. The chemicals were purchased from a chemical supply house and are of reagent grade.

Methods

All control and experimental chicks were hatched from buff White Rock eggs in commercial Montgomery Ward incubators, modified for electronic control of temperature within $\pm 0.5^{\circ}\text{C}$. Humidity was kept con-

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stant. Both temperature and humidity were continuously monitored and recorded during incubation by electronic recorders. The eggs were turned 3 times each 24-hour period. The sugar, saccharin, and cyclamate solutions were made with tap water and the strength of the solution determined on a weight/volume basis. The concentration of the sugar was standardized at 8% and 16%, sodium cyclamate at 0.91%, the calcium cyclamate at 1.81%, and sodium saccharin at 0.23%. These concentrations have been noted in the literature and are well above threshold for all animals that have been tested. The reasons for the choices of values have been discussed elsewhere (9).

Control chicks were hatched from eggs incubated at the optimal temperature of 37.5°C for 21 days. Experimental chicks were placed in a nonoptimally high temperature incubator at a temperature of 41°C for the first 72 hours of incubation, after which they were placed in the normal temperature incubator until hatching. After hatching, the chicks were banded and placed in heated starting battery brooders, eight or nine chicks per group. The solutions were presented in a watering trough with three removable waterproof sections containing water and the various concentrations of sweeteners (see reference 10 for additional details of presenting solutions and measuring solution intake). Table 1 shows the experimental paradigm.

Measurement of daily intake was made in Class A graduated cylinders to the nearest milliliter, and the volume recorded was the total intake for each group of chicks. The solutions were discarded after measurements, and fresh solutions were introduced from the refrigerated stock.

Commercial starting mash was available to the chicks at all times. The chicks were completely isolated from other animals and humans, except for those periods necessary to rotate the sections and to measure and change the solutions. Humidity and temperature were kept constant in the battery room since this facility had no windows (zone air-conditioning). Light-dark conditions were made to match those of the normal day-night at this time of the year. The experiments were conducted during July and August.

Results

Since chicks were housed in groups (for practicality as well as the necessity of rearing infant chicks in a warm environment), measurement of individual intake and preference was precluded. Because of this, the analyses of variance were more limited in scope since the interaction variances had to be used as error terms instead of within and between subject variability, and all interpretations would, therefore, tend to be conservative estimates of actual differences.

The data for the analyses are per cent intake, and for graphical presentation are mean per cent intake with the standard error of the mean as a vertical line to indicate variability. Per cent intake was used since this method of computation equates for greater bodyweight in

TABLE 1. *Experimental Paradigm.*

Age	Solution Choices					
	Water 8% glucose 0.91% Na Cyc	Water 16% glucose 0.91% Na Cyc	Water 8% glucose 1.81% Ca Cyc	Water 16% glucose 1.81% Ca Cyc	Water 8% glucose 0.23% Na Saccharin	Water 16% glucose 0.23% Na Saccharin
4 Days old	Exp. 3 Group I (n=8)	Exp. 3 Group II (n=8)		Exp. 3 Group III (n=8)		
17 Days old			Exp. 4 Group II (n=8)		Exp. 4 Group III (n=8)	Exp. 4 Group I (n=8)
30 Days old	Exp. 1 Group I (n=9)	Exp. 1 Group II (n=9)	Exp. 1 Group III (n=9)			
30 Days old	Exp. 2 Group I (n=9)	Exp. 2 Group II (n=9)	Exp. 2 Group III (n=9)			

In each cell there are two groups: Control group; Experimental (heat-stressed) group; each group was tested for eight days, 24 hrs/day, combinations in the empty cells were not made.

older chicks and, hence, greater fluid intake by presenting relative intake instead of absolute intake.

Replication: Experiment 1 vs. Experiment 2 (30-day-old chicks)

There were no significant differences in preference upon replication (Fig. 1).

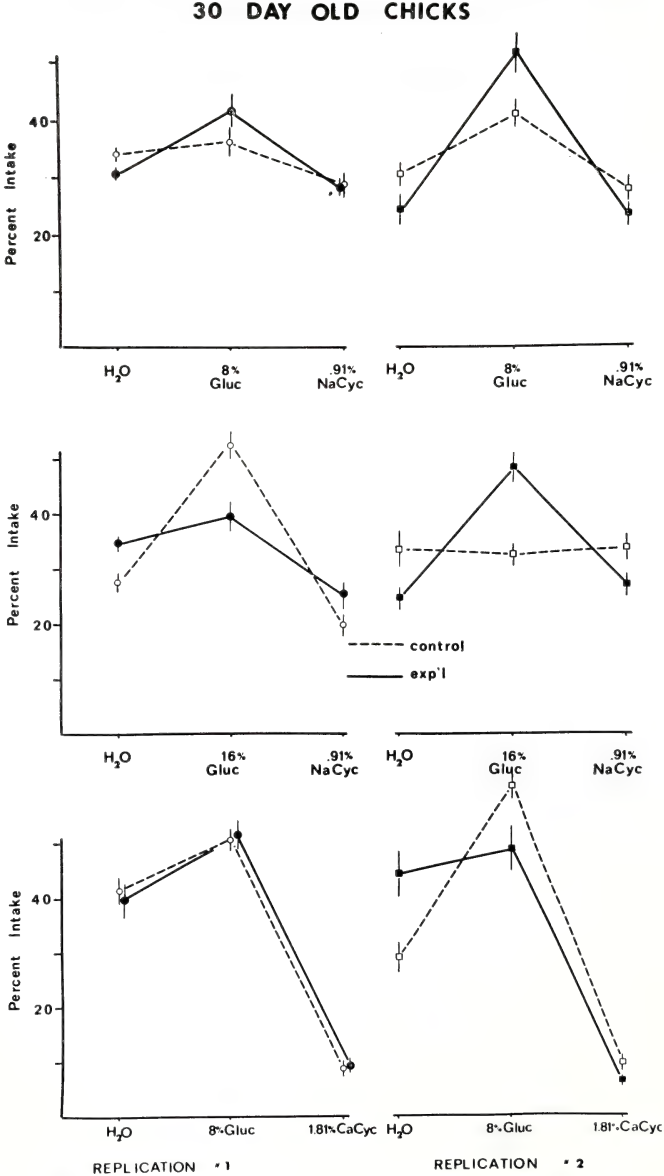


FIGURE 1. *Experiment 1 (replication 1) vs. Experiment 2 (replication 2); Group I for each replication at top of figure, Group II next, and Group III at bottom of figure.*

Experiments 1 and 2, Group I (water; 8% glucose; 0.91% sodium cyclamate) vs. Group II (water; 16% glucose; 0.91% sodium cyclamate) showed no overall difference between the two conditions of having differing glucose concentrations available. Glucose was significantly preferred to both water and cyclamate ($F = 42.089$; $df = 2/2$; $p < .025$) regardless of its relative concentration (see Table 1 for distinctions relating to Experiments 1 through 4 and Groups I through III).

In Experiments 1 and 2, Group I (water; 8% glucose; 0.91% sodium cyclamate) vs. Group III (water; 8% glucose; 1.81% calcium cyclamate) there were no overall intake differences in preferences which could be traced to the two different salts or concentrations of cyclamate. Eight per cent glucose was preferred to water or cyclamate regardless of cyclamate salt or concentration ($F = 21.50$; $df = 2/2$; $p < .05$).

Experiment 3 (4-day-old chicks)

Four-day-old chicks did not discriminate between water, 16% glucose and 0.91% sodium cyclamate (Group II), nor were their preferences different from those chicks which received water, 8% glucose and 0.91% sodium cyclamate. However, when the choices were different cyclamate salts and concentrations (Group II: water, 16% glucose; 0.91% sodium cyclamate vs. Group III: water; 16% glucose; 1.81% calcium cyclamate) overall analysis indicated that cyclamate was not preferred ($F = 1,350.959$; $df = 2/2$; $p < .001$), heat-stressed chicks preferred glucose to cyclamate ($F = 66.81$; $df = 2/2$; $p < .025$), and chicks having 1.81% calcium cyclamate as a choice preferred glucose and avoided cyclamate to a greater extent than chicks having 0.91% sodium cyclamate as an alternative ($F = 1,218.143$; $df = 2/2$; $p < .001$) (Fig. 2.)

Experiment 4 (17-day-old chicks)

Seventeen-day-old chicks preferred glucose (either 16% or 8%) to water or saccharin (Groups I and III) ($F = 24.44$; $df = 2/2$; $p < .05$). If the choices involved cyclamate or saccharin (Groups II and III respectively) chicks preferred glucose to either water or the artificial sweetener ($F = 78.37$; $df = 2/2$; $p < .025$) and further, Group III chicks drank relatively more saccharin (and less 8% glucose) and Group II chicks drank relatively less cyclamate (glucose II vs. glucose III, $t = 4.373$; $df = 14$; $p < .001$; saccharin III vs. cyclamate II, $t = 4.19$; $df = 14$; $p < .001$) (Fig. 2).

Age Differences

Thirty-day-old chicks preferred glucose to either water or sodium cyclamate while four-day-old chicks were indiscriminate (water; 16% glucose; 0.91% sodium cyclamate; $F = 36.513$; $df = 2/2$; $p < .05$). Any differences between 30-day-old and 4-day-old chicks when presented with choices of water, 8% glucose, and 0.91% sodium cyclamate were not due to experimental manipulation, but were the results of excessive variability.

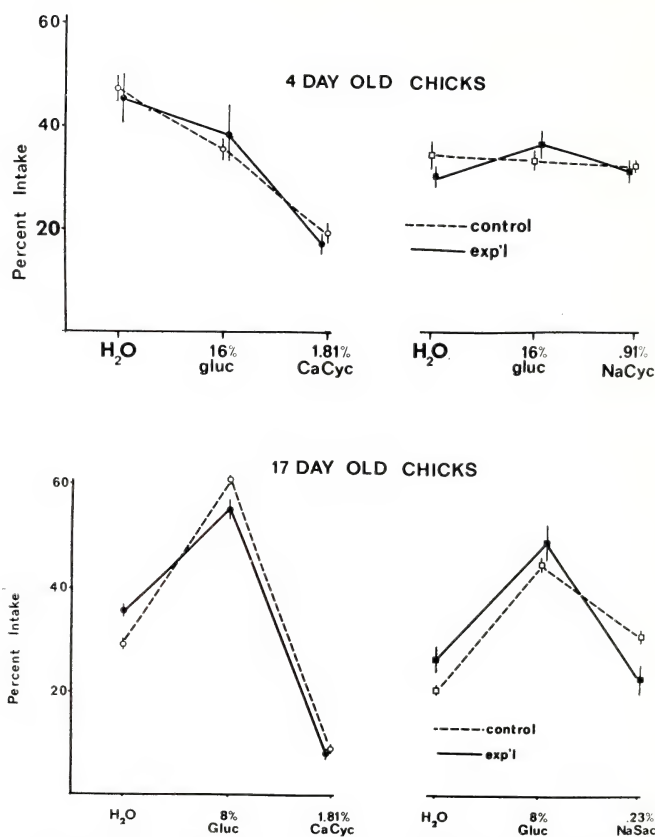


FIGURE 2. Experiments 3 (top) and 4 (bottom); only Group III, left and Group II, right (Exp. 3) and Group III, right and Group II left (Exp. 4) compared in each experiment.

Seventeen and 30-day-old chicks were both given choices of water, 8% glucose, and 1.81% calcium cyclamate. For all chicks glucose intake was greatest and cyclamate least ($F = 2,403.959$; $df = 2/2$; $p < .001$). Heat-stressed chicks drank relatively more water, less glucose and equal small amounts of cyclamate—still preferring glucose, however, ($F = 35.325$; $df = 2/2$; $p < .05$), and finally, the younger chicks (17 days old) showed a more pronounced glucose preference ($F = 26.435$; $df = 2/2$; $p < .05$) (Fig. 3).

Discussion

Wagner and Gunther (10) previously have shown that chicks can discriminate various taste stimuli, preferring some over water, while avoiding others. The present studies clearly reinforce this.

Chicks consistently avoid cyclamates, the sole exception being 4-day-old chicks which were indiscriminate to a 16% glucose or 0.91%

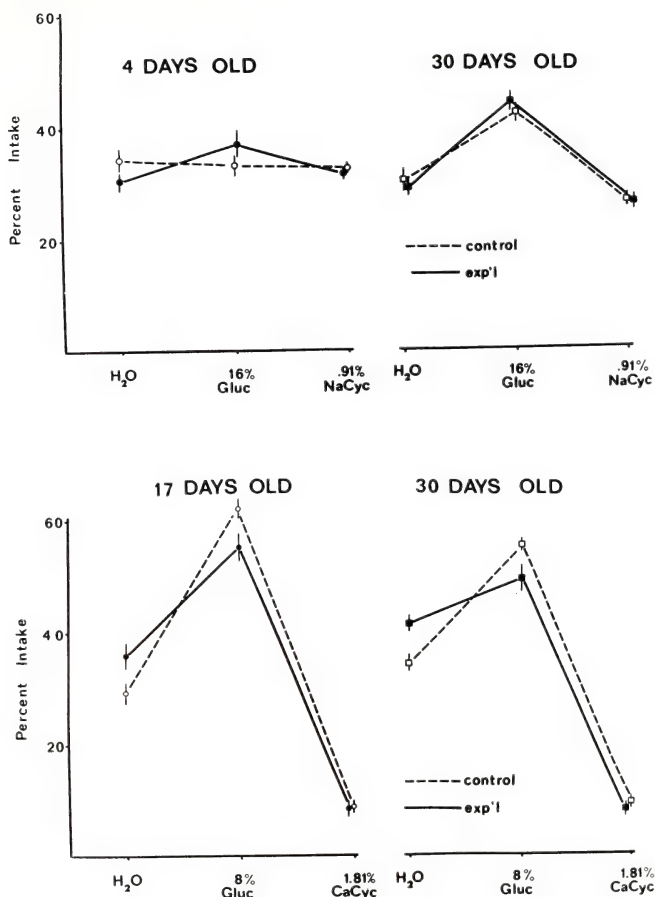


FIGURE 3. Age differences in intake of water and sweeteners.

sodium cyclamate. Are chicks avoiding cyclamates because of the suspected toxicity of cyclamate, or because of some taste-quality inhere in cyclamate solutions? Dr. Jacqueline Verrett (unpublished research progress reports, U.S. Food and Drug Adm.) found that cyclamate injected into incubating eggs produced embryonic deformities, but it is difficult to generalize from such studies to food preferences or aversions (6). Chicks reject toxic lactose, but the aversion develops gradually (10). In the present study, the nonintake of cyclamate was immediate, suggesting that taste rather than toxicity accounts for the aversion. This same phenomenon has been observed in rodents (7, 8, 9).

A comparison of rodent and chick preferences and aversions for sweeteners does not imply that these two species inhabit similar sensory worlds in general. However, the universality of the chemical sense and its function in food selection might lead one to expect some

similarities in taste preferences for simple sweeteners. Since, in nature, sweets are a source of calories basic to the energy requirements of all living creatures, especially warm-blooded animals, it is quite logical to make comparative analyses of sweet preferences and aversions across diverse species with some expectation of finding similarities.

While chicks never preferred the saccharin to glucose, comparative data from rats and mice would suggest that this should not be expected since rats and mice prefer 8% glucose to 0.23% sodium saccharin (8, 9).

Perhaps a better test of relative palatability of the two artificial sweeteners is the observation that saccharin was drunk to a much greater extent than 1.81% cyclamate under similar conditions by 17-day-old chicks.

The effects of heat-stress during incubation are overshadowed by preference differences because of age and type of choice available. In fact, heat related differences might be considered equivocal since heat-stressed 4-day-old chicks drank relatively less water and more glucose than their normal counterparts, whereas heat-stressed 17-day-old and 30-day-old chicks drank relatively more water and less glucose than their normal counterparts. Previously cited research also found stress differences to be less pronounced than preference differences because of type of sugar choice available.

Acknowledgment

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